

The molecular ecology and management of Steller sea lions



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Steller sea lion population genetic studies

Genetics 101

Steller sea lion genetics: the three stock hypothesis

Dispersal patterns and population structure in Steller sea lions

Emigration, migration and colonization in Steller sea lions: new rookeries suggest a new paradigm

Kinship, mating systems and colonization in Steller sea lions

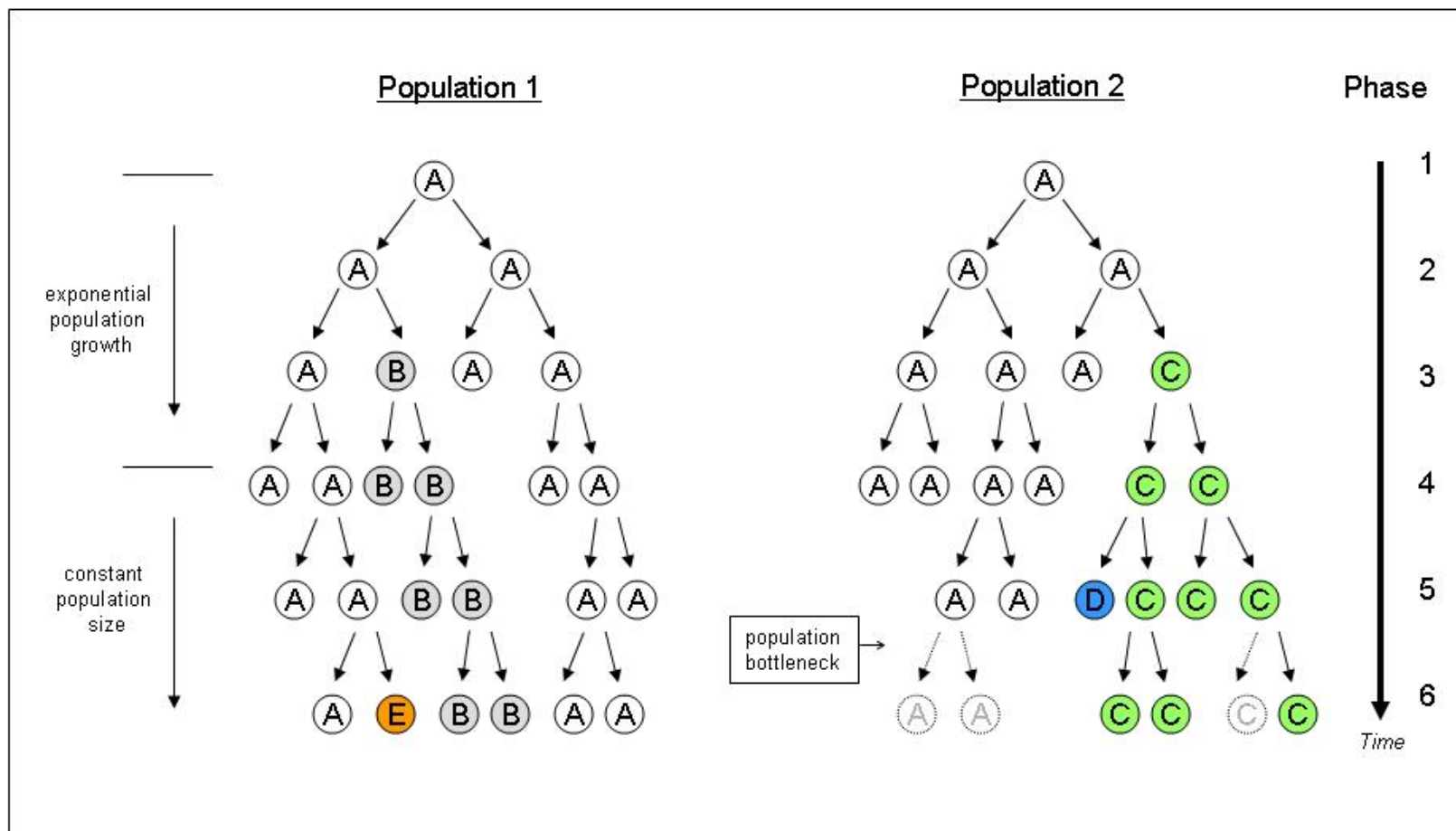


Population Genetics and Marine Mammal Management

The study of inheritance and patterns of genetic variation within and between populations, and the evolutionary forces that determine these patterns: mutation, genetic drift, gene flow, and selection

Mutation:	the process by which new variation is produced
Gene flow:	the exchange of variants among groups of organisms via dispersal and interbreeding
Genetic drift:	the loss of variation over time due primarily to differences in survival and reproductive success among individuals within a population
Selection:	a deterministic relationship between how free a genetic locus is allowed to vary and how essential its function is to an individual's fitness

Heredity and the forces that shape mtDNA variation within and among populations



Haploid marker: e.g., mtDNA

Patterns of variation in **neutral** genetic markers are influenced by 3 primary factors.....

gene flow	– dispersal, interbreeding	d, m
genetic drift	– population size	N_e
mutation	– generation time	μ

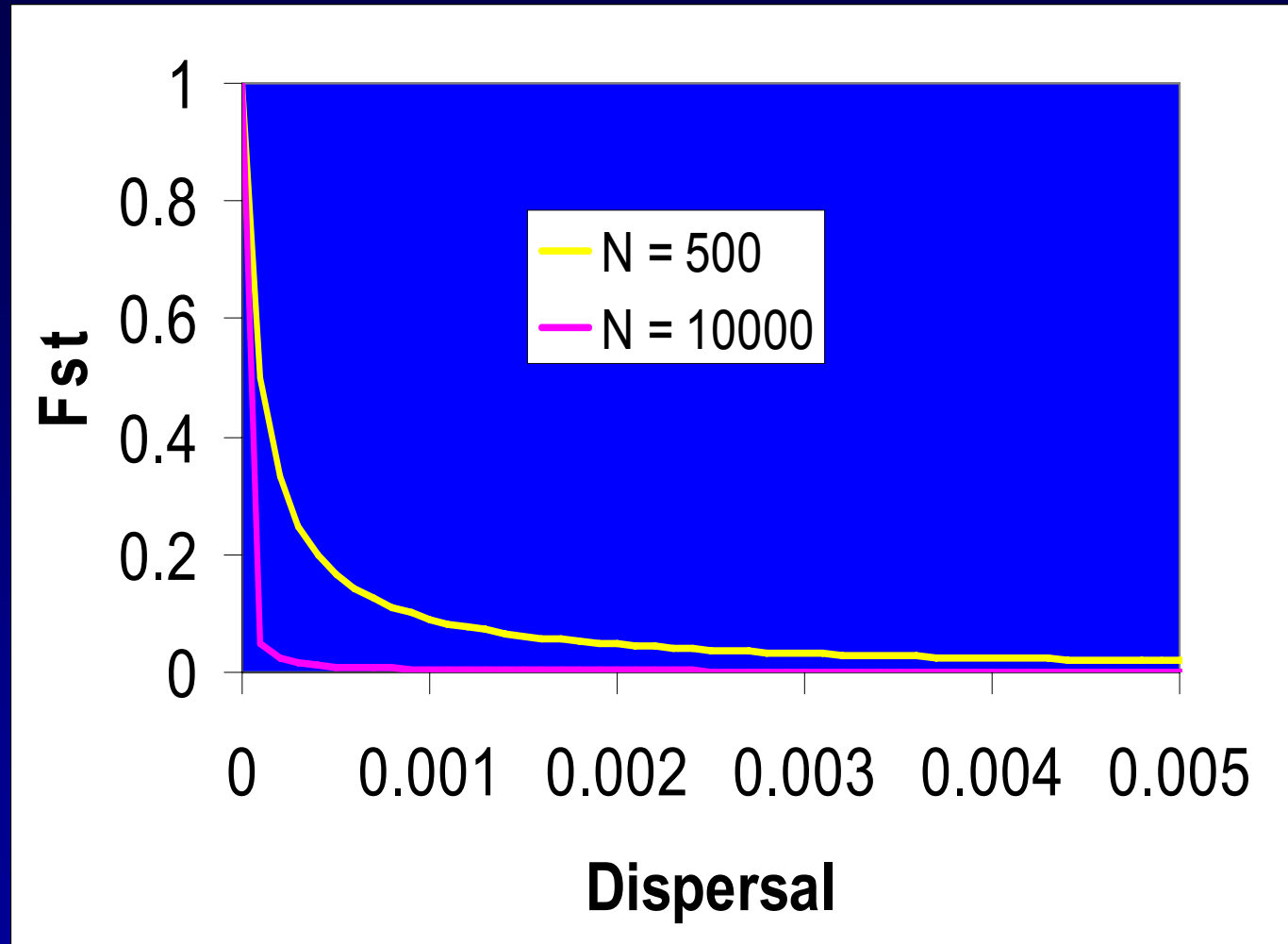
Over ecological timescales the effects of mutation are likely to be small

Wright's Island model

mtDNA

$$F_{st} = \frac{1}{2Nm + 1}$$

$$F_{st} = 1/(2Nm + 1) = 1/(2NdT + 1)$$



Population genetic studies on Steller sea lions

Gene flow and dispersal on contemporary timescales

identify management units
extinction-colonization, metapopulation dynamics
conduct population viability analysis

Gene flow and dispersal over evolutionary timescales

identify management units
estimate evolutionary significance

Kinship, mating systems and social organization

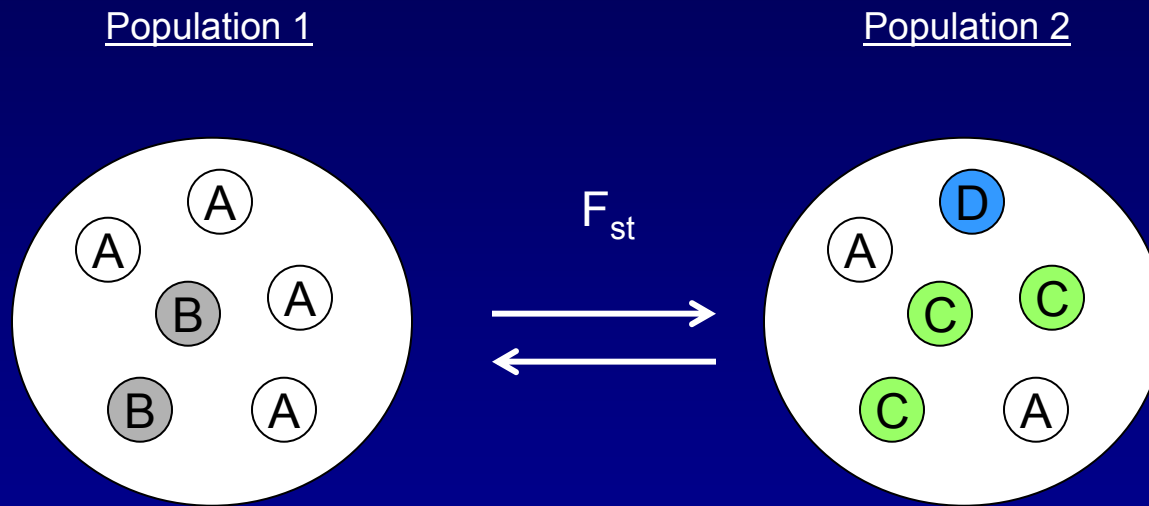
estimate N_e
estimate level of inbreeding
estimate lifetime reproductive success

Genetic diversity and population history

loss of genetic diversity, genetic 'bottlenecks'
evolutionary potential
genetic health

Gene flow and dispersal on contemporary timescales

identify management units
extinction-colonization, metapopulation dynamics
conduct population viability analysis



Wright's island model

$$F_{st} = \frac{1}{2N_e m + 1}$$

Limitation: current models assume equilibrium

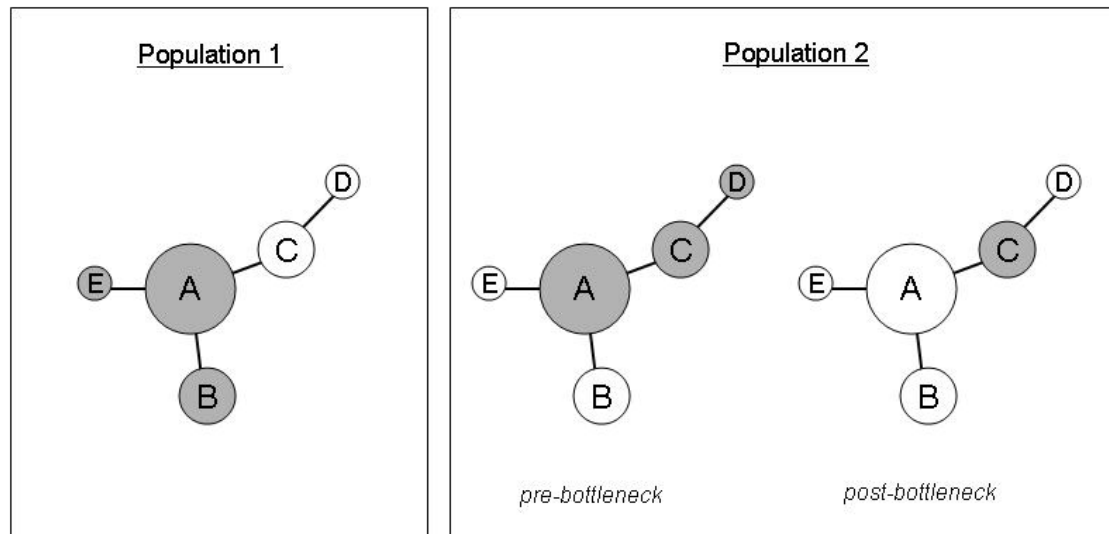
Gene flow and dispersal over evolutionary timescales

Phylogeography

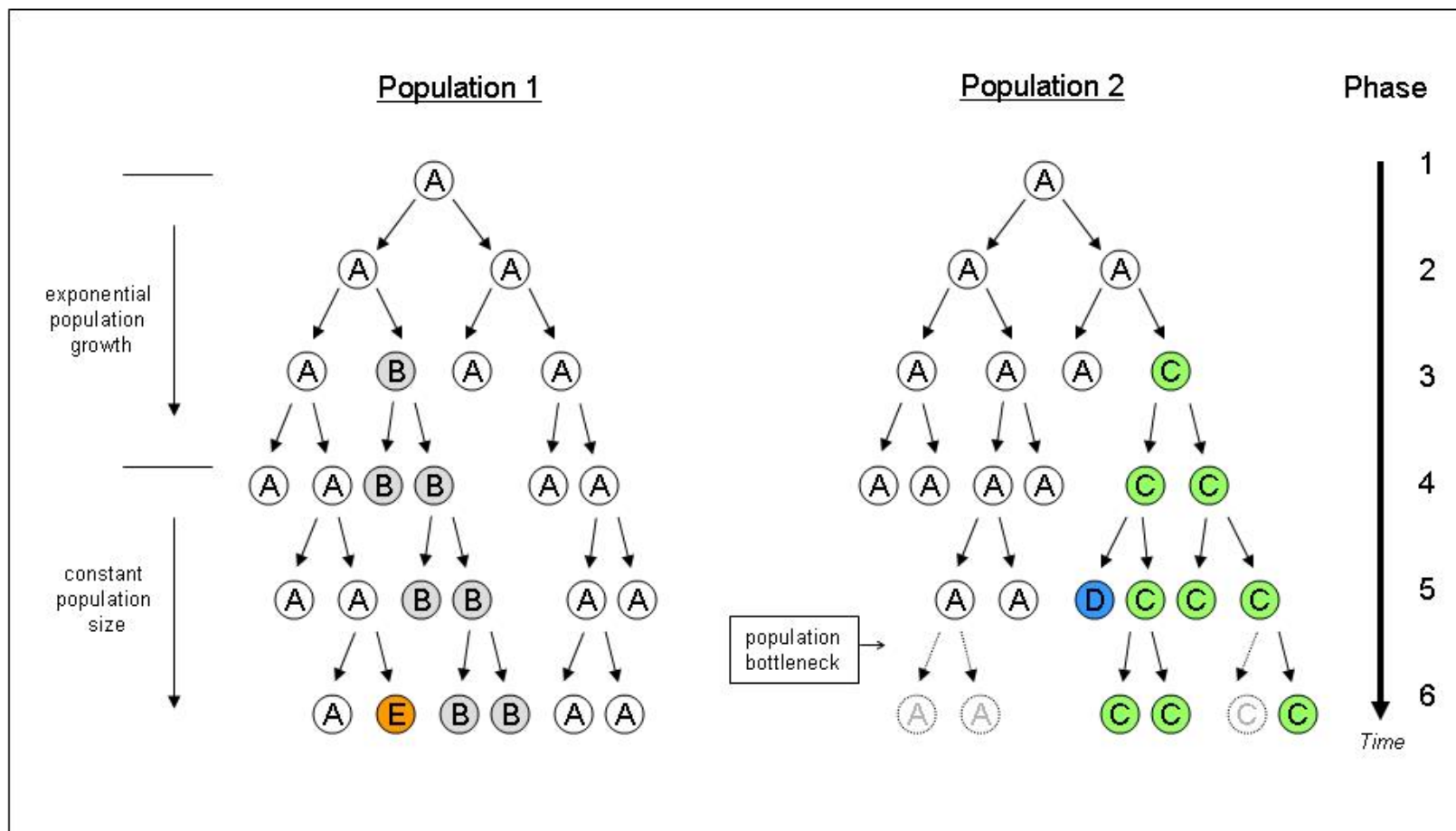
identify management units
estimate evolutionary significance

Haplotype	DNA sequence
A	CGTTACGATAGACC
B	CGT A ACGATAGACC
C	CGTTAC C ATAGACC
D	CGTT T CATAGACC
E	CGTTACGATA C ACC

Population expansion
A is ancestral haplotype
B and E are unique to Pop 1
C and D are unique to Pop 2
Evolutionary distinct populations

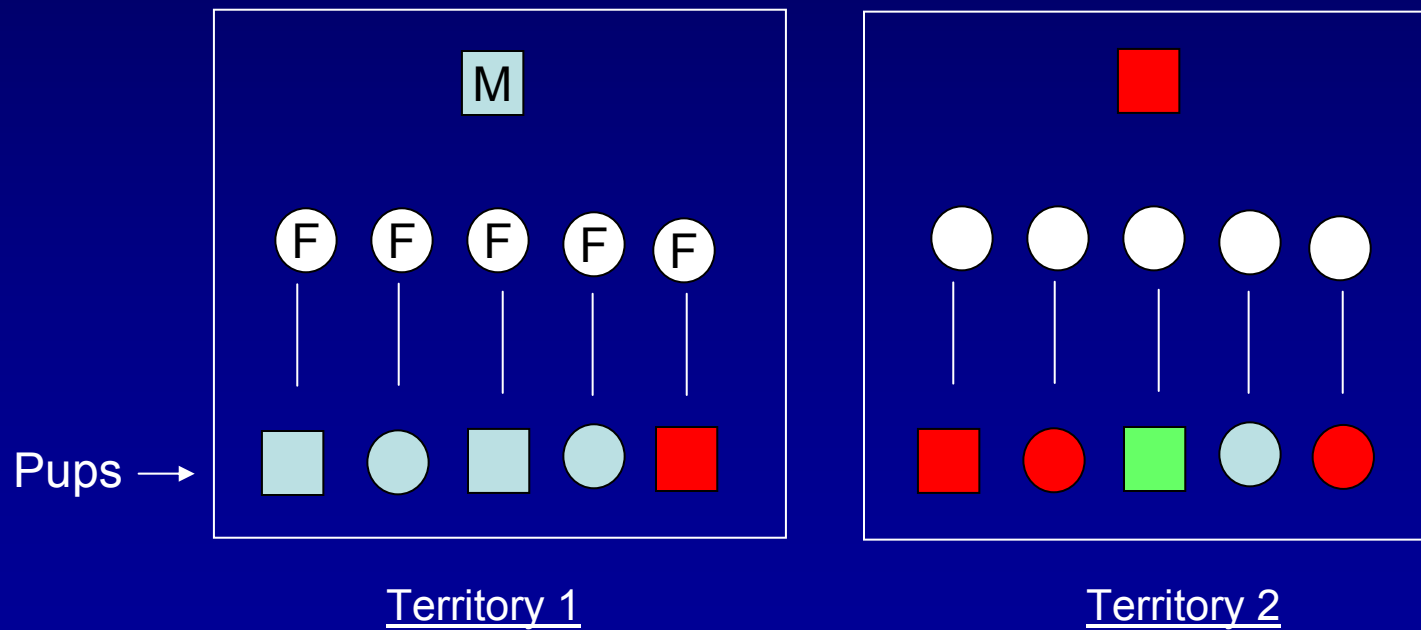


Heredity and the forces that shape mtDNA variation within and among populations



Kinship, mating systems and social organization

Ne
random mating, level of inbreeding
philopatry to natal group
estimate lifetime reproductive success



Polygynus species: low N_e

Molecular genetic studies on Steller sea lions



K. Raum-Suryan

Management objectives
Generating hypothesis
Sample collection
Marker choice
Interdisciplinary collaborations

Multiple markers
mtDNA
microsatellite (STR) loci

Cloning technology
PCR
Sequence analysis
Fragment length analysis

Phylogeny reconstruction
↓
Individual ID



The management objectives.....

Endangered Species Act, 1973

Prevent the extinction of 'species', 'subspecies' and 'distinct population segments' of fish, wildlife and plants - preserve biodiversity and evolutionary potential

Marine Mammal Protection Act, 1972

Maintain 'species' and 'population stocks' as significant functioning elements of their ecosystem, and not allow them to diminish below optimum sustainable population levels

.....determines the definition of management unit or stock

*ESA – no gene flow, evolutionarily distinct
ecological and biological significance*

*MMPA – limited female dispersal
demographically distinct*

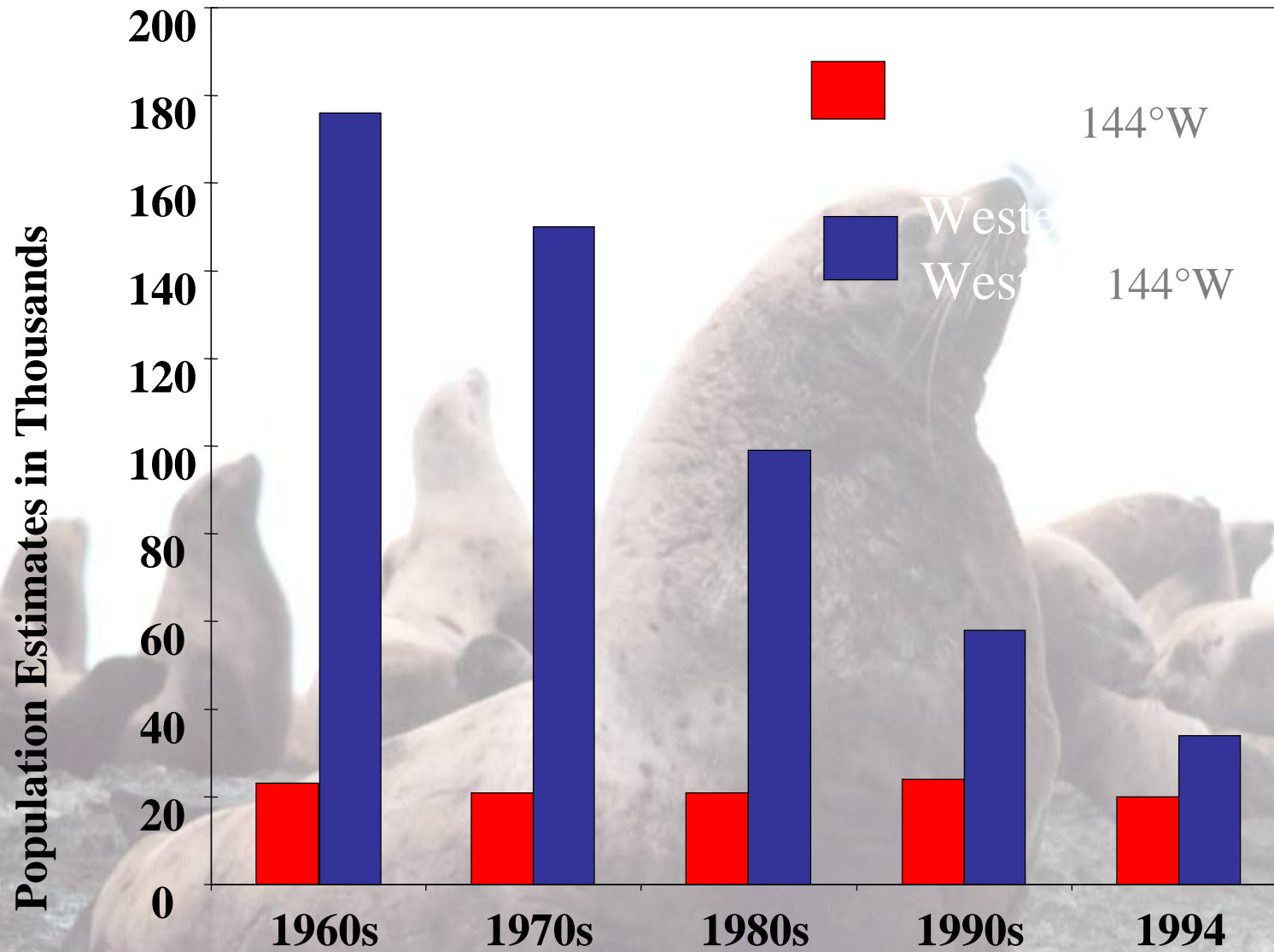
Steller sea lion genetics ———

————— the three stock hypothesis

John Bickham

Texas A&M University





**Population Estimates for Steller Sea Lions
(1960-1994)**

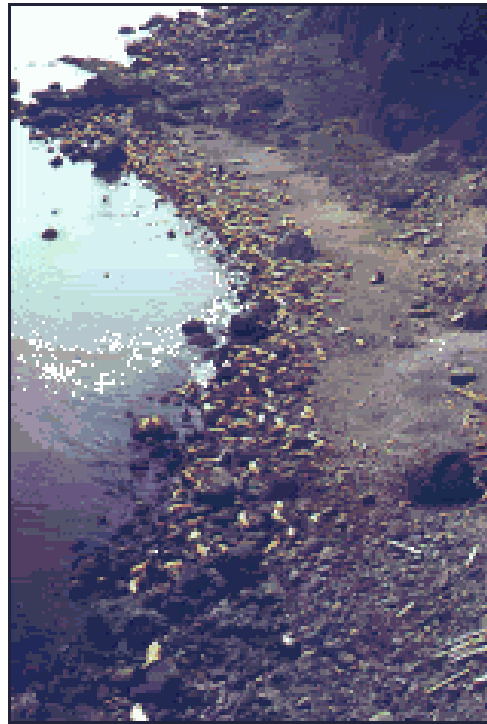
Population Trends

- The total SSL population numbered between 240,000 and 300,000 during the late 1950's and early 1960's.
- Only 116,000 were estimated range-wide in 1989.

Pictures from Ugamak Island rookery, Eastern Aleutian Islands



-1969-



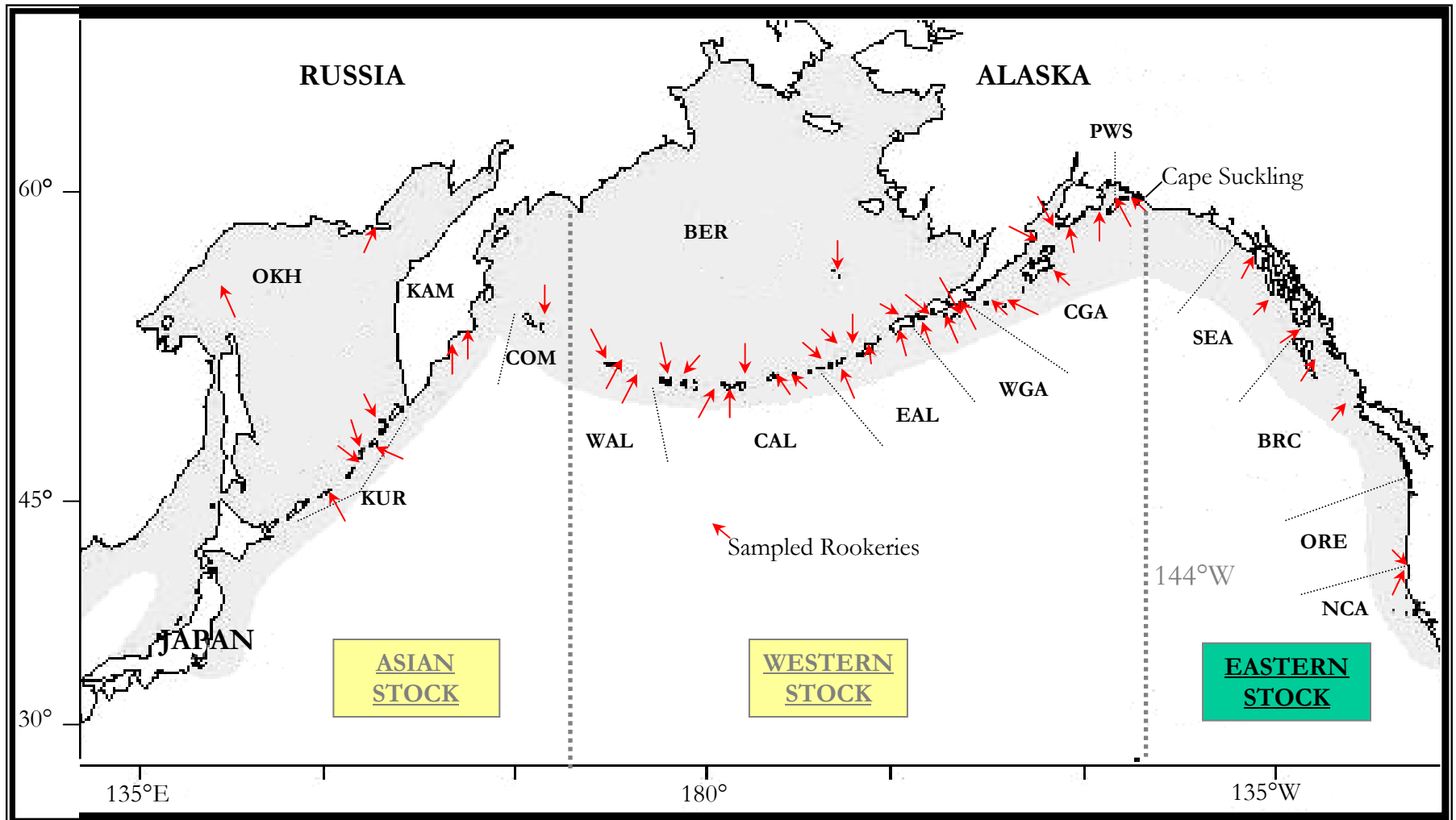
-1979-



-1989-

A Third Stock?

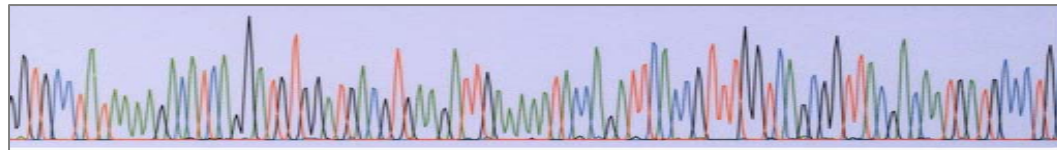
- Previous research suggests that animals sampled from Russian rookeries are genetically distinct from other western stock sea lions.



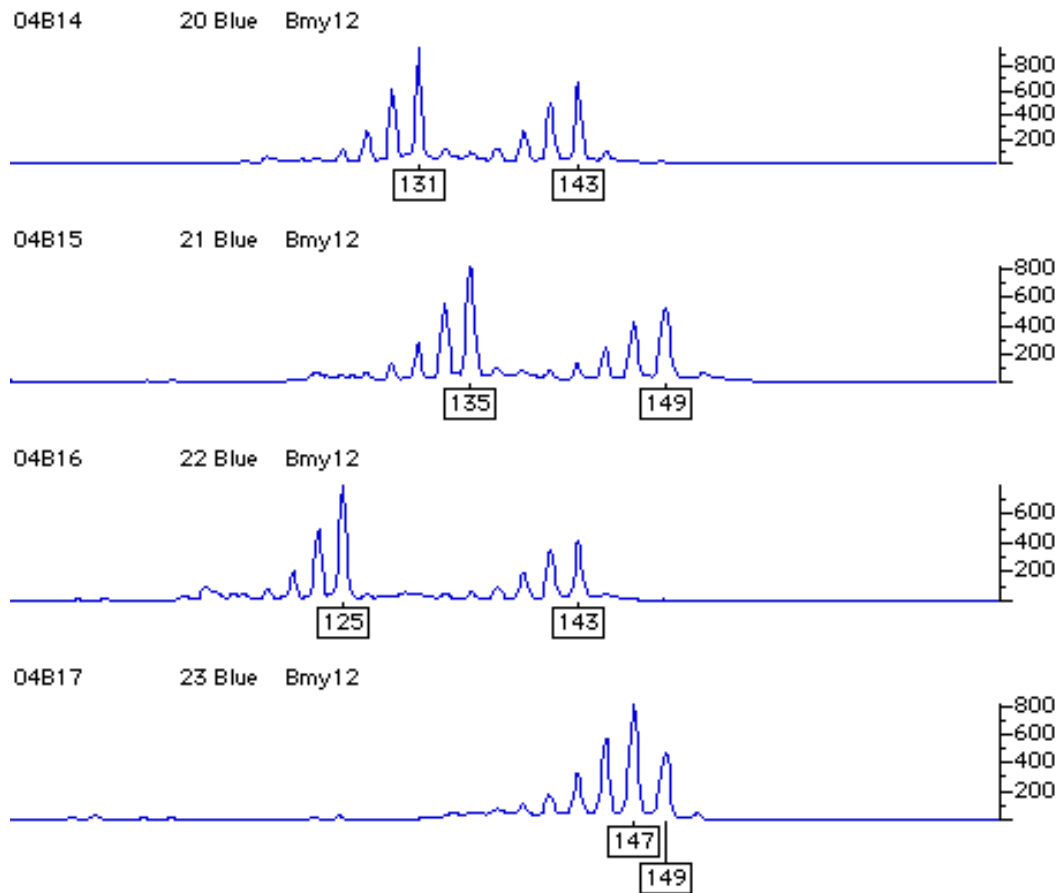
Methods

Why mtDNA?

- ✓ maternally inherited
- ✓ undergoes no recombination
- ✓ evolves 5-10 times faster than nuclear genes



- Control Region is the most rapidly evolving segment of the mtDNA and is most commonly used for population genetic studies.
- Cytochrome *b* is a commonly used marker for both inter-specific and population genetic studies.



- Microsatellites are highly polymorphic, biparentally inherited nuclear markers commonly used today for population genetic analyses.

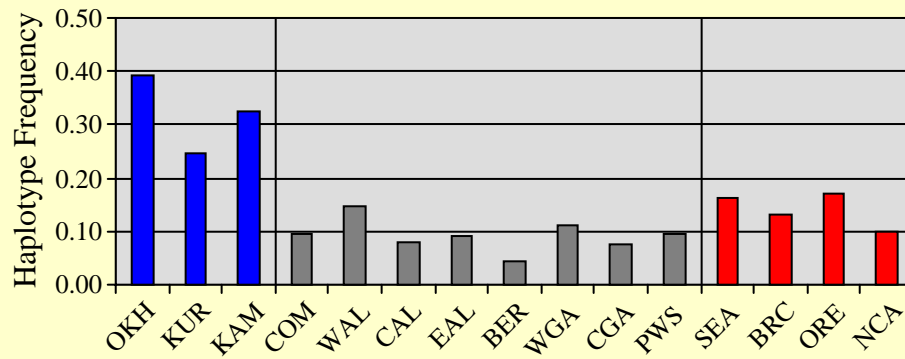
Control Region Sample Sizes



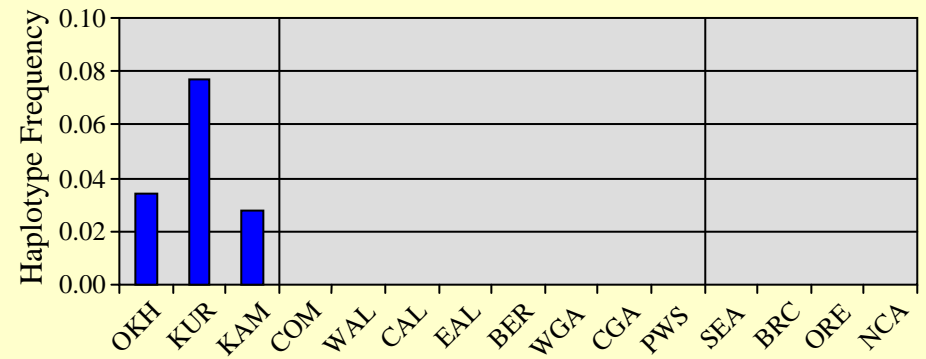
Stock	Region	Sample Size
EASTERN STOCK	NCA (1 ROOKERY)	51
	ORE (1 ROOKERY)	83
	BRC (2 ROOKERIES)	23
	SEA (3 ROOKERIES)	76
	TOTAL	233
WESTERN STOCK	PWS (2 ROOKERIES)	124
	BER (1 ROOKERY)	40
	CGA (7 ROOKERIES)	122
	WGA (5 ROOKERIES)	99
	EAL (6 ROOKERIES)	272
	CAL (9 ROOKERIES)	213
	WAL (3 ROOKERIES)	55
	COM (1 ROOKERY)	126
	TOTAL	1051
ASIAN STOCK	KAM (2 ROOKERIES)	71
	KUR (5 ROOKERIES)	207
	OKH (2 ROOKERIES)	148
	TOTAL	426
	GRAND TOTAL	1,710

Control Region Haplotype Distributions

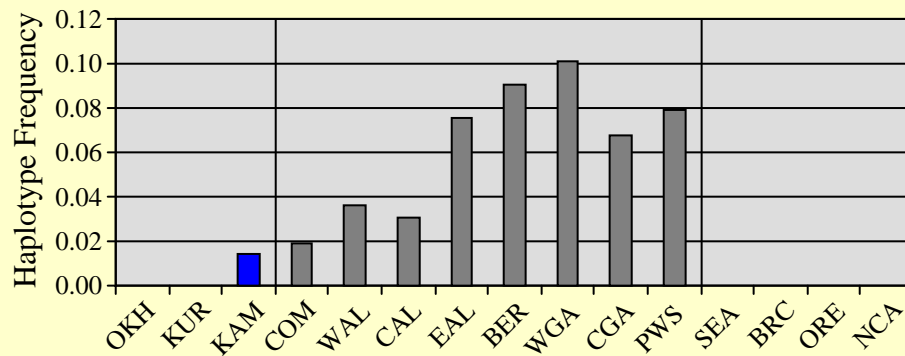
Haplotype BB



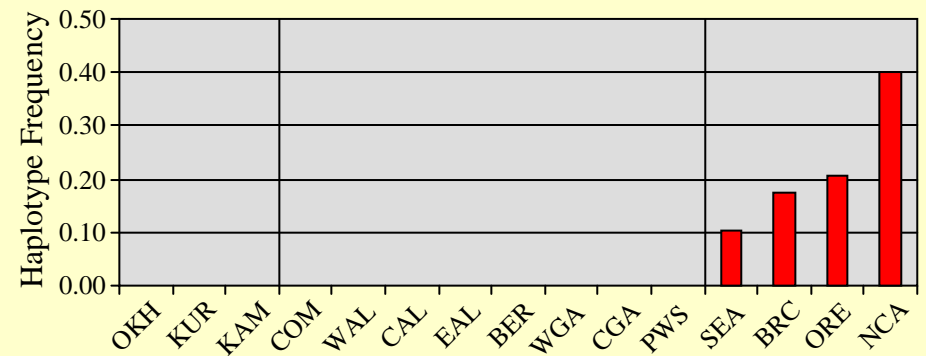
Haplotype LLL



Haplotype E

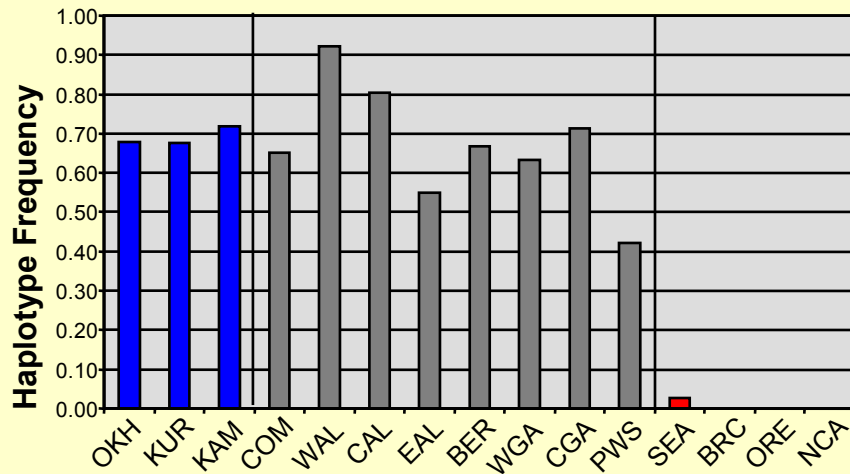


Haplotype H

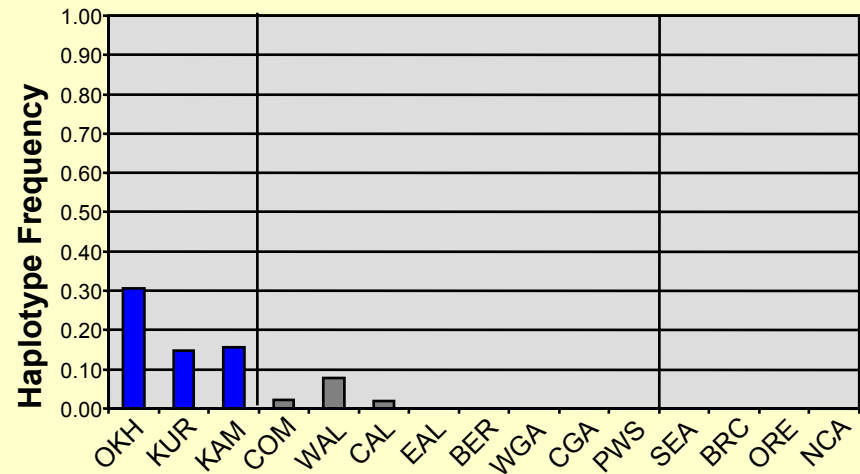


Cytochrome *b* Haplotype Distributions

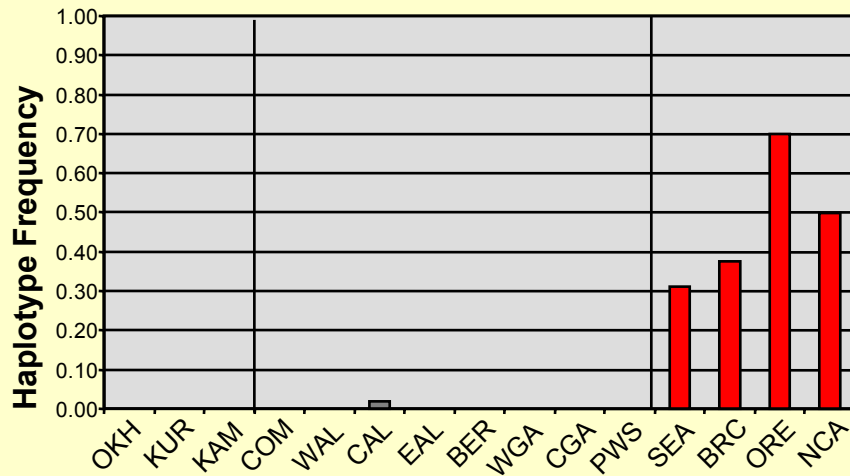
Haplotype 1



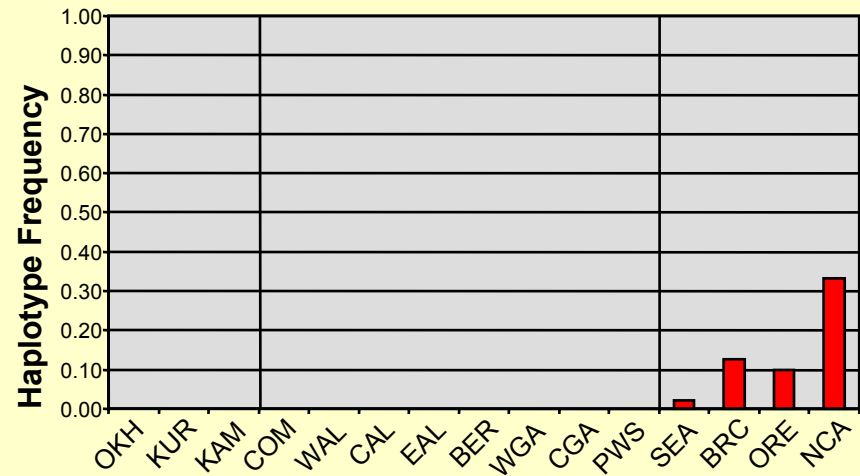
Haplotype 4



Haplotype 2



Haplotype 10



NJ Tree – Regions

Legend

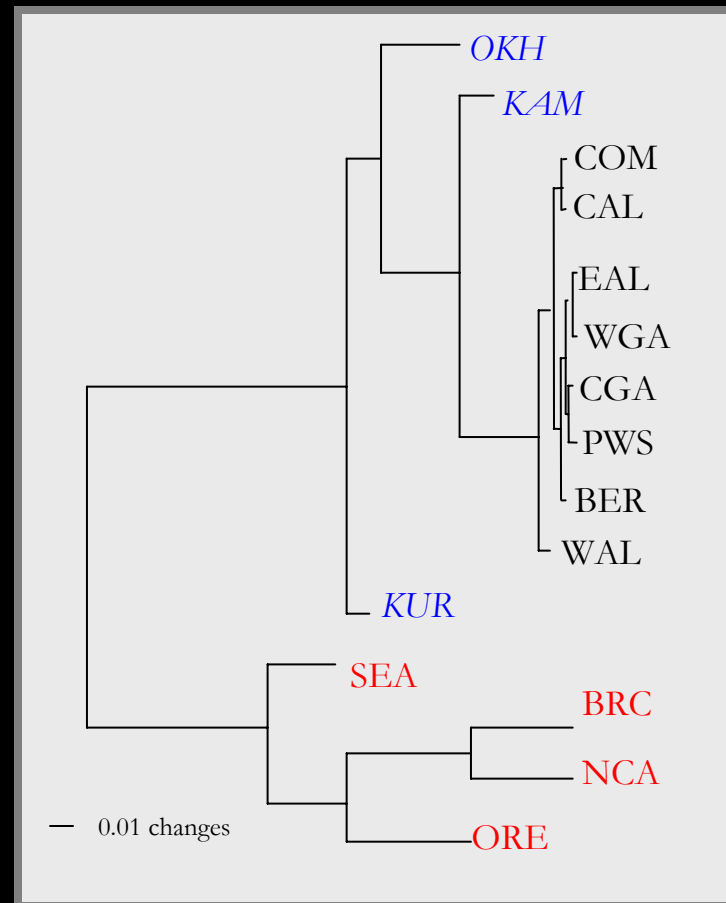
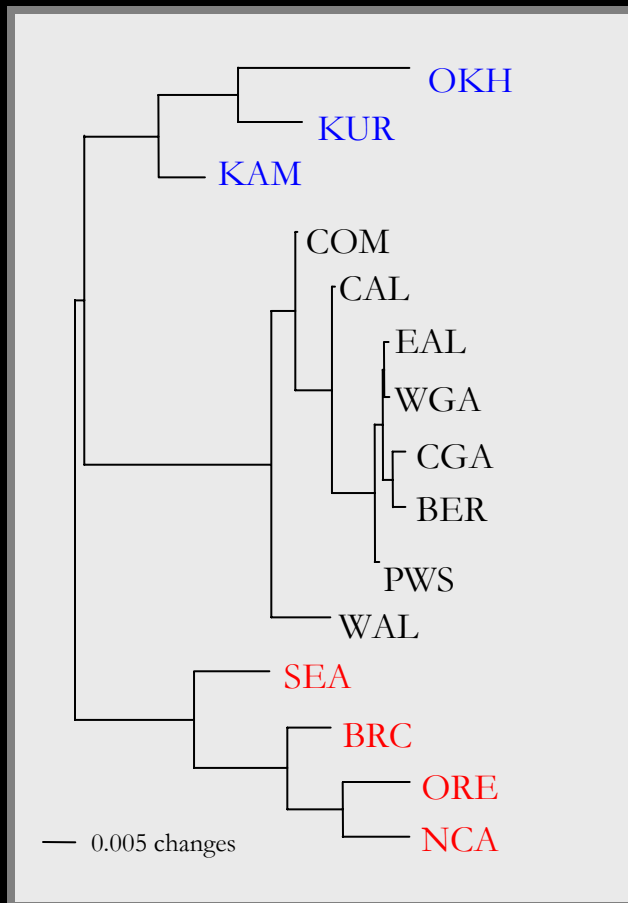
Asian Stock

Western Stock

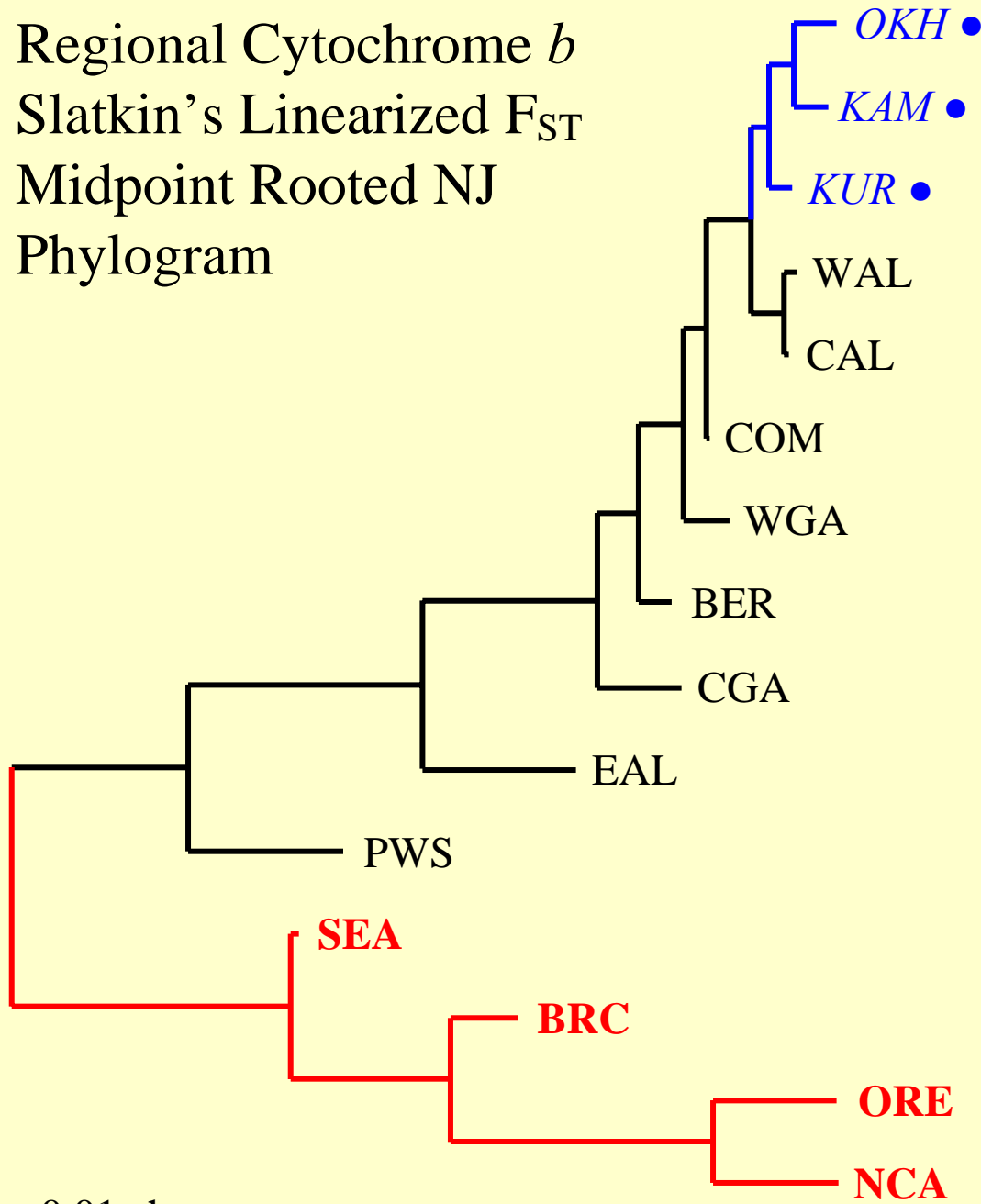
Eastern Stock

Conventional Fst Values

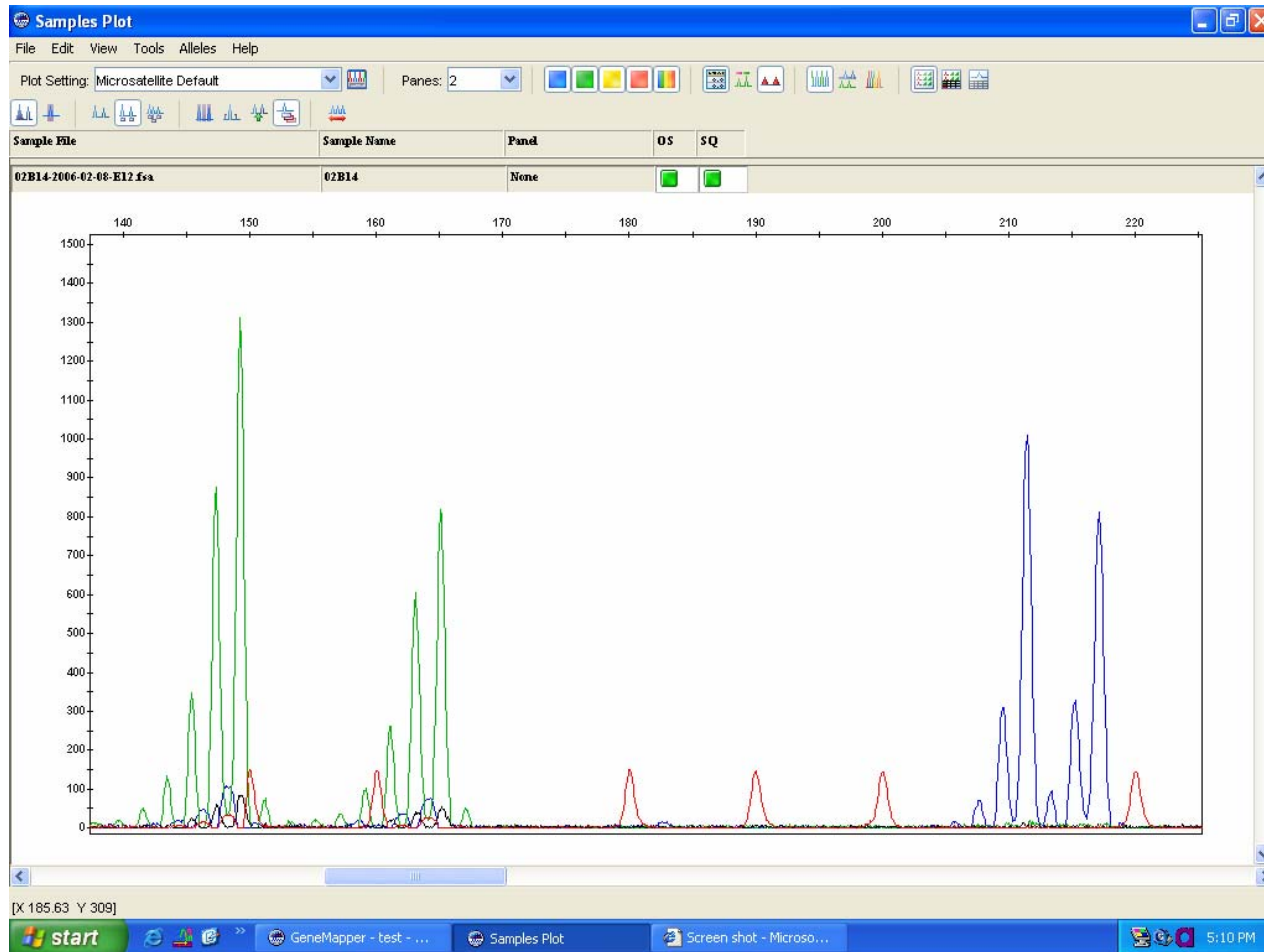
Φ_{st} Values



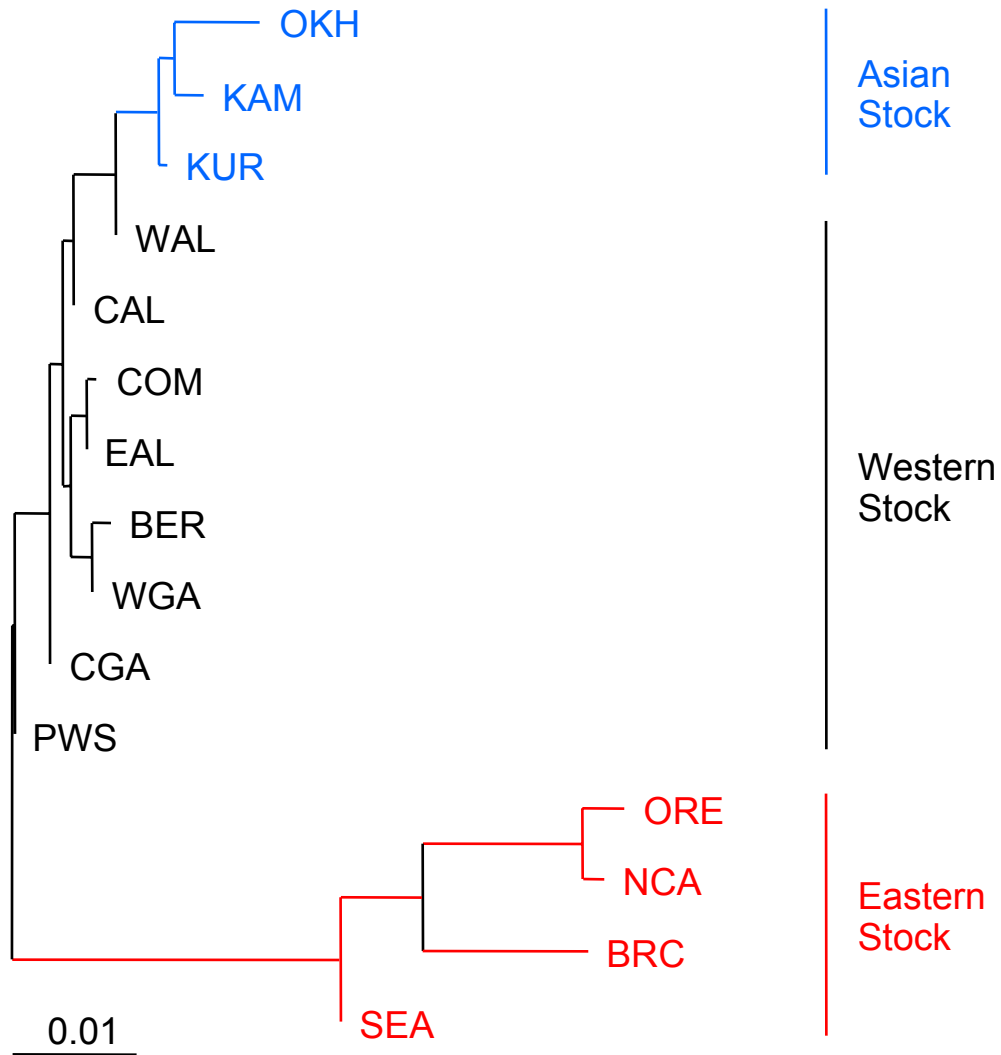
Regional Cytochrome *b*
Slatkin's Linearized F_{ST}
Midpoint Rooted NJ
Phylogram



- 0.01 changes



NJ Phylogram of Regions Based on Microsatellite Data.



Conclusions

Control Region Phylogeography

- Despite the generally high sample sizes in this study, resolution of population substructure increased when rookeries were grouped into their respective regions.
 - Possible reason – sample size effect

Rookeries

- Eastern stock rookeries were clearly differentiated from all other rookeries when using Φ -statistics.
- Differentiation of eastern stock rookeries was less clear when using F-statistics.

Regions

- F-statistics resolved three lineages corresponding to the Eastern, Western and Asian stocks.
- Φ -statistics resolved only two lineages, although Asian regions clearly occupied the most basal positions of the Asian/Western lineage.

Conclusions

Cytochrome *b*

Population Genetics

Cytochrome *b* has considerably less variability than Control Region.

A high number of first position mutations and subsequent amino acid substitutions could result from selection.

Phylogeography

Cytochrome *b* has less homoplasy and a more completely resolved haplotype network than Control Region.

There is good support for the separation of the eastern and western stocks.

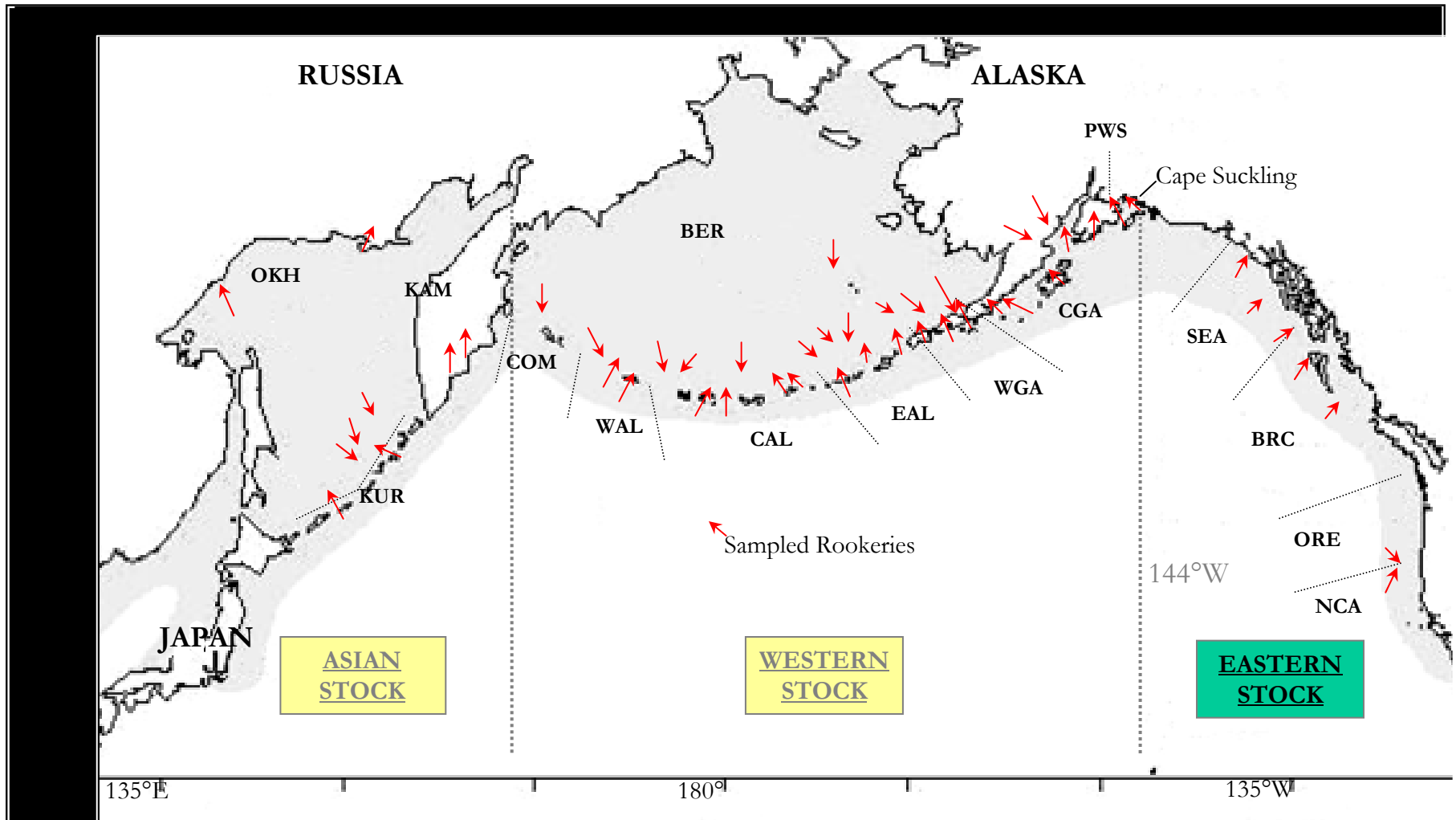
There is little support for the separation of the Asian and western stocks.

Conclusions

Microsatellites

- There is good support for the separation of the eastern and western stocks.
- There is little support for the separation of the Asian and western stocks.
- Further studies are planned to determine if the recognition of two subspecies, one to include the eastern stock and the other to include the western and Asian stocks, is appropriate.

The recognition of three management units and two subspecies may be appropriate for Steller sea lion conservation.



Study 1. **Dispersal patterns and population structure in Steller sea lions**

Topic: Population Structure
Dispersal behavior
Marine ecosystems

Key elements:

mtDNA
Phylogeography
Demographic independence
Stock Identification
Oceanography
Behavioral ecology
Sample size
Data integration



G. O'Corry-Crowe, B.L. Taylor, T. Gelatt, T. Loughlin, J. Bickham, M. Basterretche, K. Pitcher, and D.P. DeMaster (in review.)

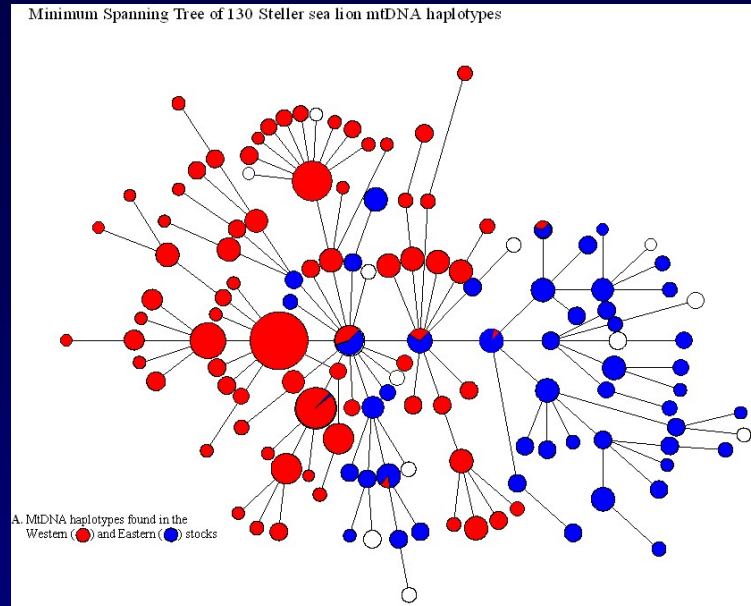
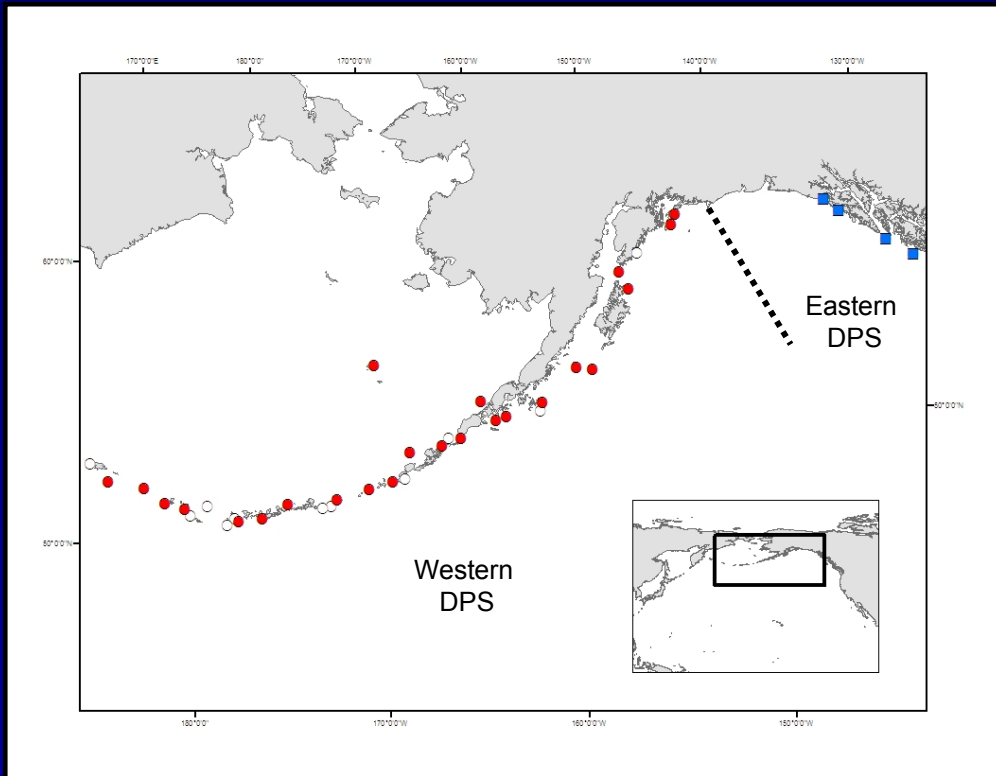


Supported by NOAA Fisheries and the Alaska Department of Fish and Game



Population Structure and dispersal patterns in Steller Sea lions

Marker type: mtDNA
531bp
n=1,654
28 rookeries



Substantial phylogeographic partitioning of mtDNA haplotypes among long established **Eastern DPS** and **Western DPS** rookeries confirming an ancient divergence between these two populations

Using a range of coalescent models of historical population growth as priors in a bayesian MCMC analysis of mtDNA sequences and substitution rates based on a 2myr age of divergence between *Eumetopias* and *Zalophus*, the time of divergence of lineages found exclusively in the Eastern or Western DPS today, was estimated at 226,000 (CV. 36,000-479,000) to 399,000 (CV. 240,00-544,000) yr. ago. Average pairwise differences among individuals from both DPSs date population divergence at 213,000 yr.

Subdivision and dispersal within the **Western DPS**

F_{st} for mitochondrial DNA

Gulf of Alaska

E. Aleutians

Central Aleutians

W. Aleutians

n =	S. Rocks 57	Wooded 47	Sugarloaf 64	Marmot 67	Chirikov 56	Chowiet 24	Atkins 56	Pinnacle 51	Clubbing 25	Ugamak 93	Akutan 78	Amak 53	Yunaska 34	Seguam 46	Kasatochi 55	Adak 65	Gramp R. 56	Ayugadak 20	Kiska 39	Buldir 30	Agattu 40	
S. Rocks	0.006																					
Wooded	-0.006	0.007																				
Sugarloaf	-0.007	0.011	-0.007																			
Marmot	-0.001	0.023	-0.004	-0.006																		
Chirikov	-0.011	0.012	-0.010	-0.011	-0.002																	
Chowiet	0.001	0.015	-0.005	0.002	-0.002	0.001																
Atkins	-0.003	0.015	0.000	0.003	0.000	-0.002	-0.010															
Pinnacle	0.015	0.049	0.006	-0.005	-0.004	-0.006	0.012	0.020														
Clubbing	0.003	0.019	0.005	0.007	0.004	0.004	-0.002	-0.004	0.027													
Ugamak	0.014	0.021	0.013	0.015	0.009	0.030	0.000	0.005	0.031	0.005												
Akutan	0.000	0.018	-0.001	-0.001	-0.001	-0.011	0.007	0.011	0.002	0.011	0.023											
Amak	0.016	0.046	0.025	0.023	0.018	0.032	0.018	0.011	0.058	0.011	0.015	0.046										
Yunaska	0.014	0.029	0.010	0.014	0.005	0.012	0.010	0.013	0.029	0.004	0.015	0.019	0.007									
Seguam	0.016	0.048	0.023	0.026	0.016	0.034	0.024	0.020	0.057	0.023	0.026	0.045	-0.012	0.012								
Kasatochi	0.015	0.036	0.017	0.025	0.013	0.025	0.011	0.008	0.054	0.007	0.015	0.036	-0.010	0.000	-0.006							
Adak	0.013	0.048	0.018	0.023	0.016	0.029	0.023	0.025	0.047	0.024	0.025	0.032	0.002	0.009	-0.007	0.003						
Gramp R.	0.022	0.050	0.024	0.036	0.027	0.026	0.026	0.024	0.071	0.024	0.046	0.039	0.012	0.010	0.006	0.010	0.006					
Ayugadak	0.017	0.034	0.017	0.024	0.016	0.034	0.010	0.010	0.056	0.010	0.009	0.038	-0.005	-0.002	-0.001	-0.004	0.002	0.001				
Kiska	0.036	0.054	0.034	0.041	0.028	0.041	0.023	0.023	0.064	0.018	0.022	0.055	-0.003	-0.004	0.010	-0.002	0.019	0.017	0.000			
Buldir	0.027	0.051	0.029	0.039	0.027	0.050	0.018	0.022	0.072	0.021	0.017	0.051	-0.005	0.007	0.000	0.001	0.002	0.009	-0.015	0.001		
Agattu																						

P > 0.05 : 
 0.05 > P > 0.01 : 
 P < 0.01 : 

Inter-rookery differentiation → limited female dispersal

Subdivision and dispersal within the **Western DPS**

F_{st} for mitochondrial DNA

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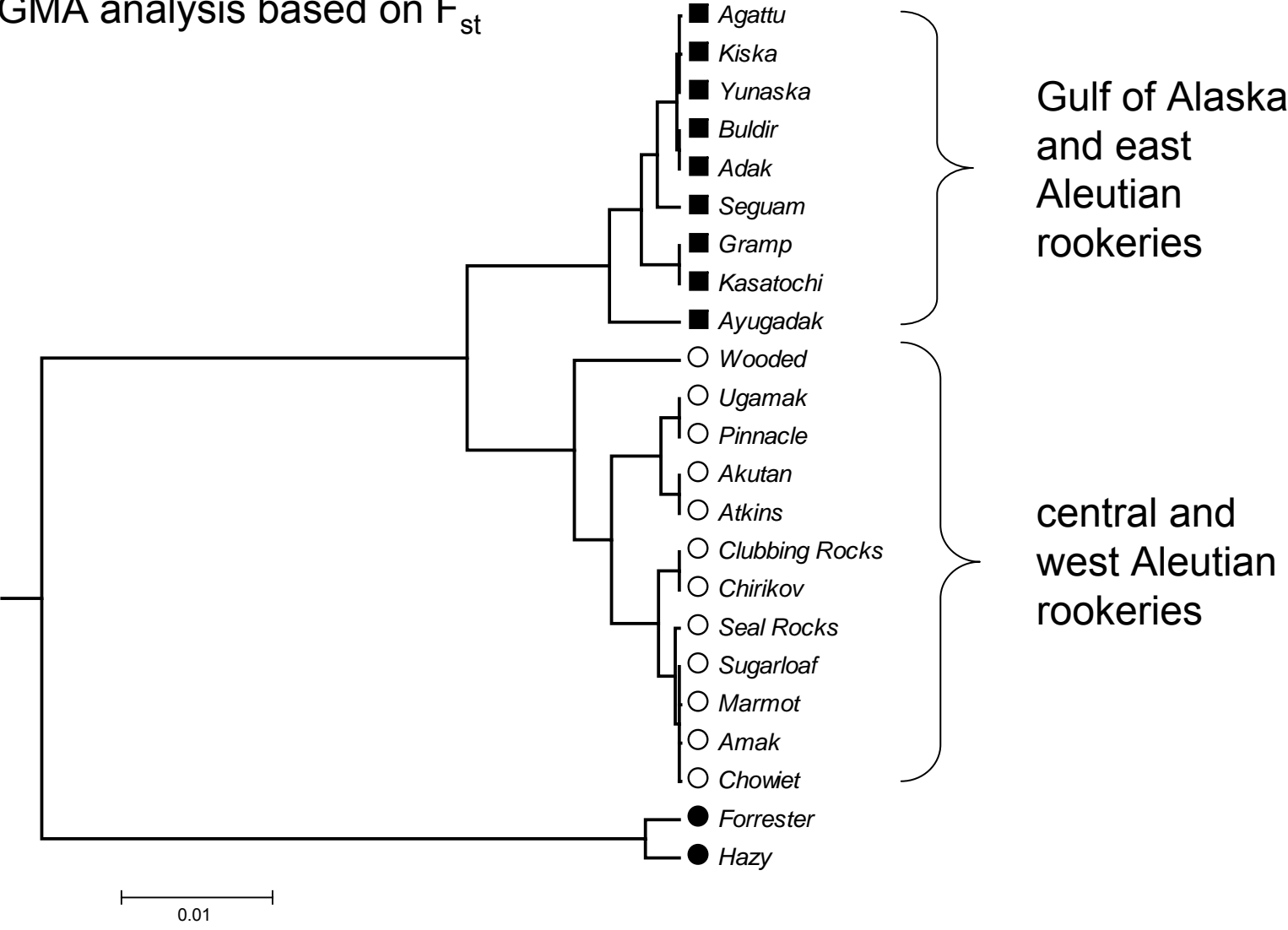
	S. Rocks	Wooded	Sugarloaf	Marmot	Chirikov	Chowiet	Atkins	Pinnacle	Clubbing	Ugamak	Akutan	Amak	Yunaska	Seguam	Kasatochi	Adak	Gramp R.	Ayugadak	Kiska	Buldir	Agattu	
<i>n</i> =	57	47	64	67	56	24	56	51	25	93	78	53	34	46	55	65	56	20	39	30	40	
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Adak	0.013	0.048	0.018	0.023	0.016	0.029	0.023	0.025	0.047	0.024	0.025	0.032	0.002	0.009	-0.007	0.003						
Gramp R.	0.022	0.050	0.024	0.036	0.027	0.026	0.026	0.024	0.071	0.024	0.046	0.039	0.012	0.010	0.006	0.010	0.006					
Ayugadak	0.017	0.034	0.017	0.024	0.016	0.034	0.010	0.010	0.056	0.010	0.009	0.038	-0.005	-0.002	-0.001	-0.004	0.002	0.001				
Kiska	0.036	0.054	0.034	0.041	0.028	0.041	0.023	0.023	0.064	0.018	0.022	0.055	-0.003	-0.004	0.010	-0.002	0.019	0.017	0.000			
Buldir	0.027	0.051	0.029	0.039	0.027	0.050	0.018	0.022	0.072	0.021	0.017	0.051	-0.005	0.007	0.000	0.001	0.002	0.009	-0.015	0.001		
Agattu																						

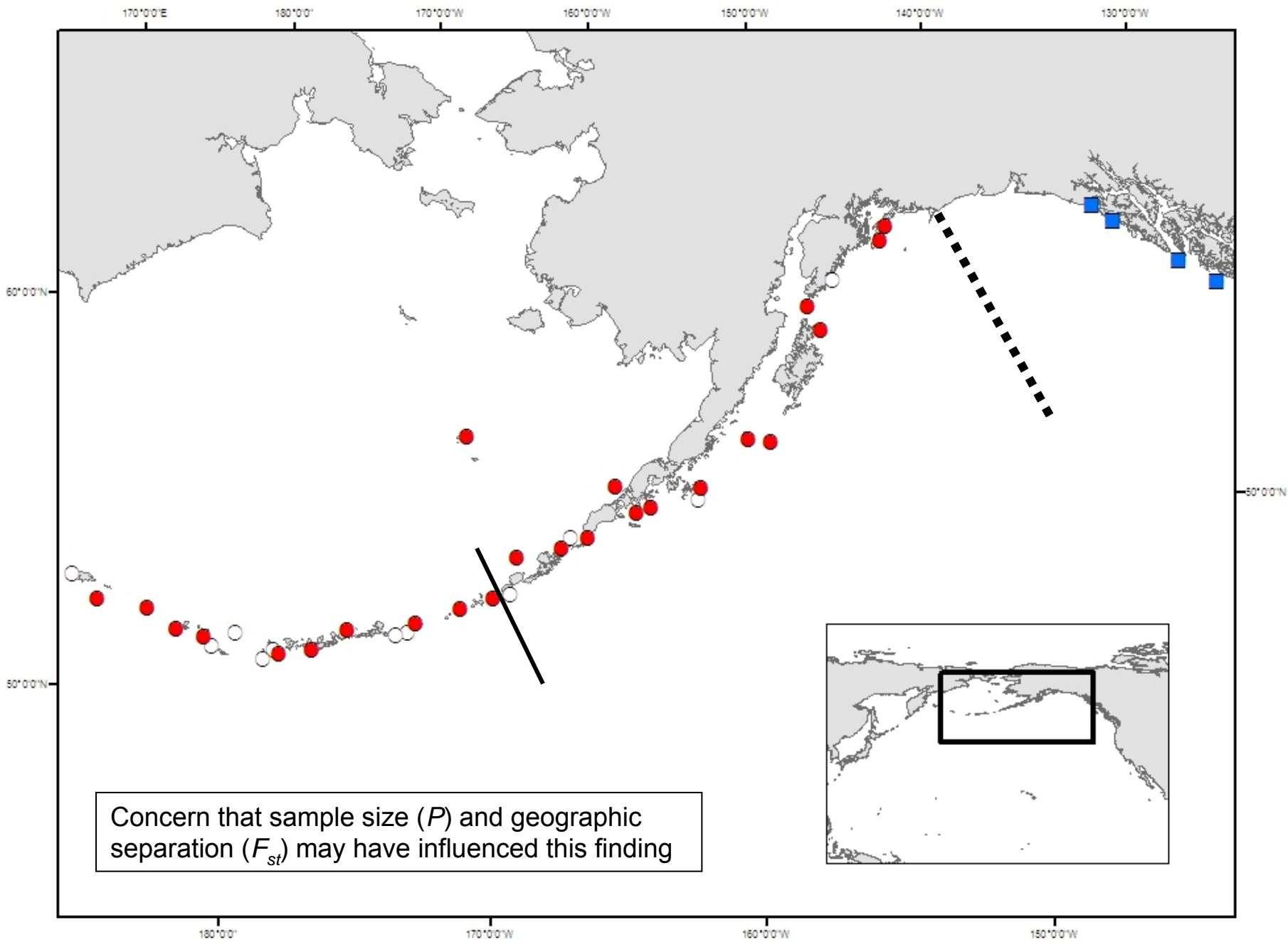
P > 0.05 : 
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 P < 0.01 : 

Inter-rookery differentiation → limited female dispersal

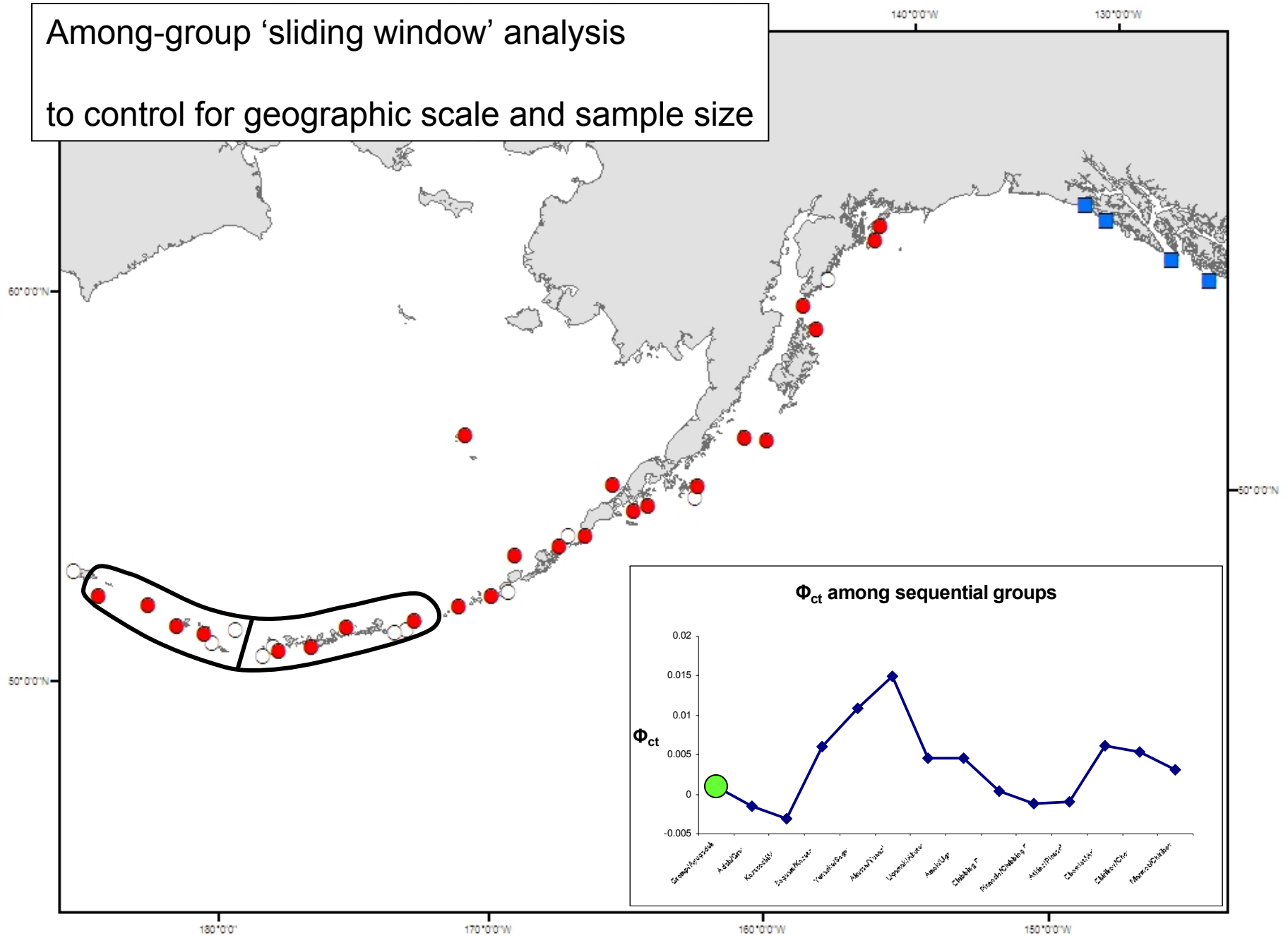
Regional differentiation → demographic independence

UPGMA analysis based on F_{st}



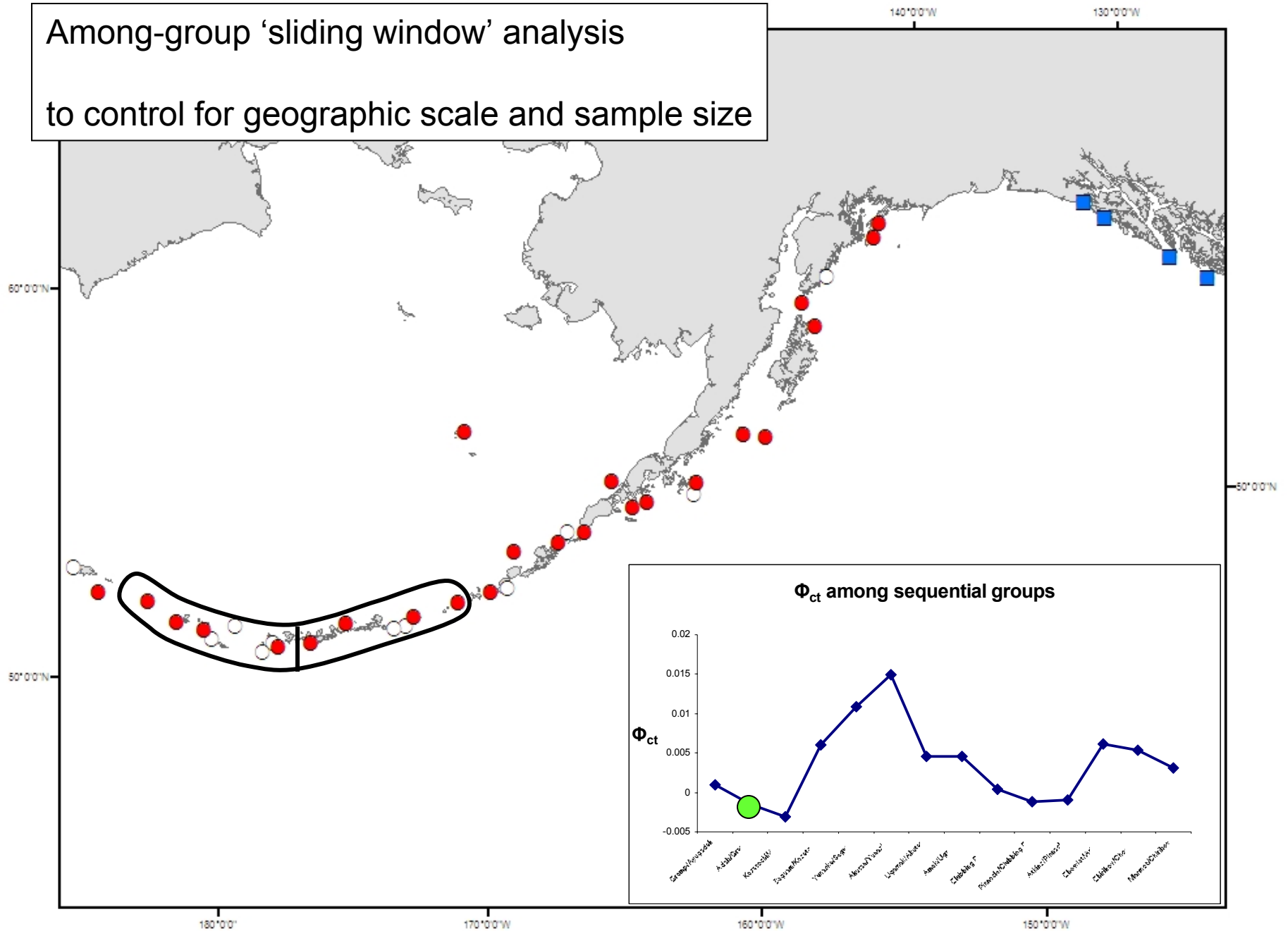


Among-group 'sliding window' analysis
to control for geographic scale and sample size

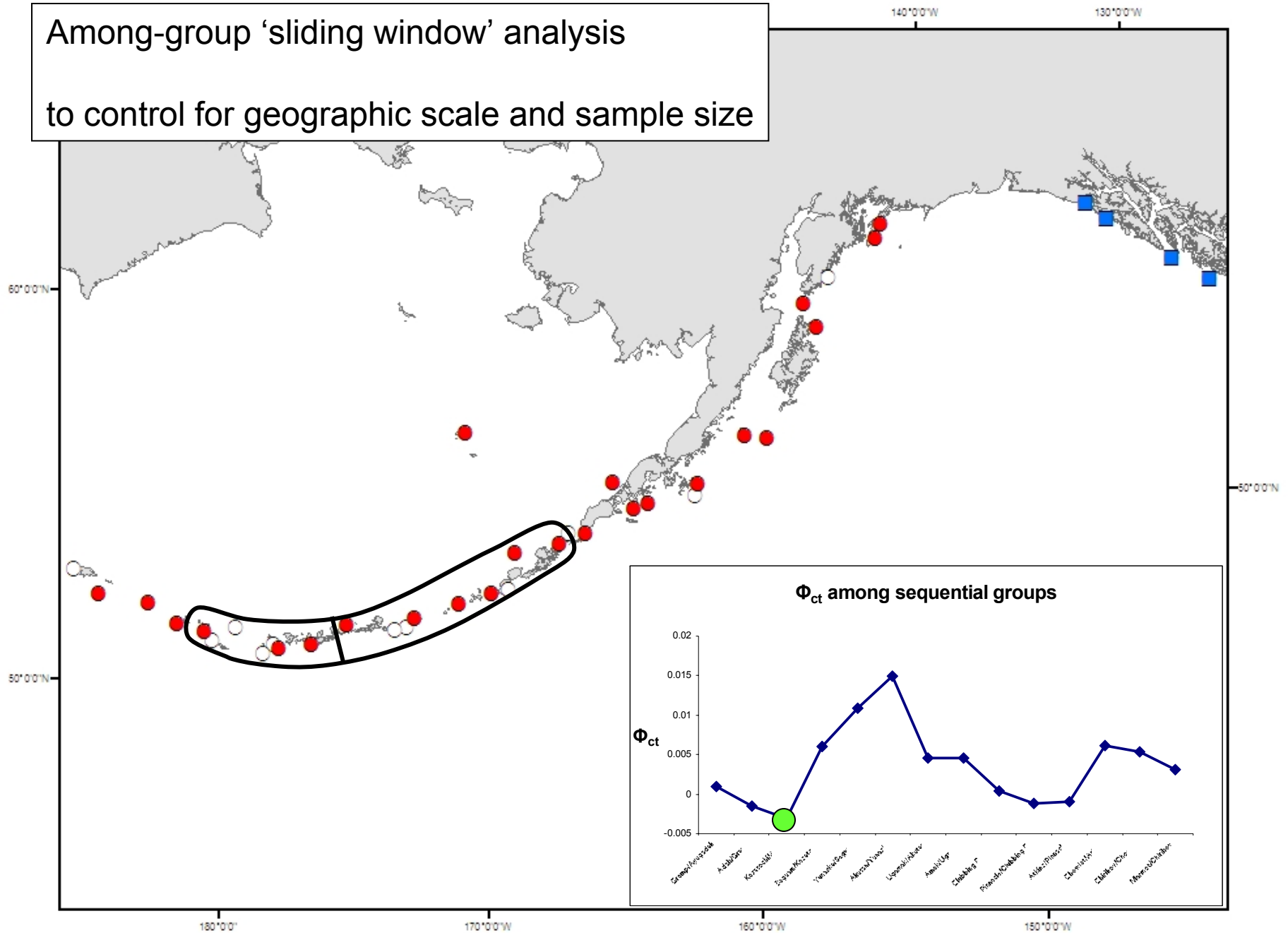


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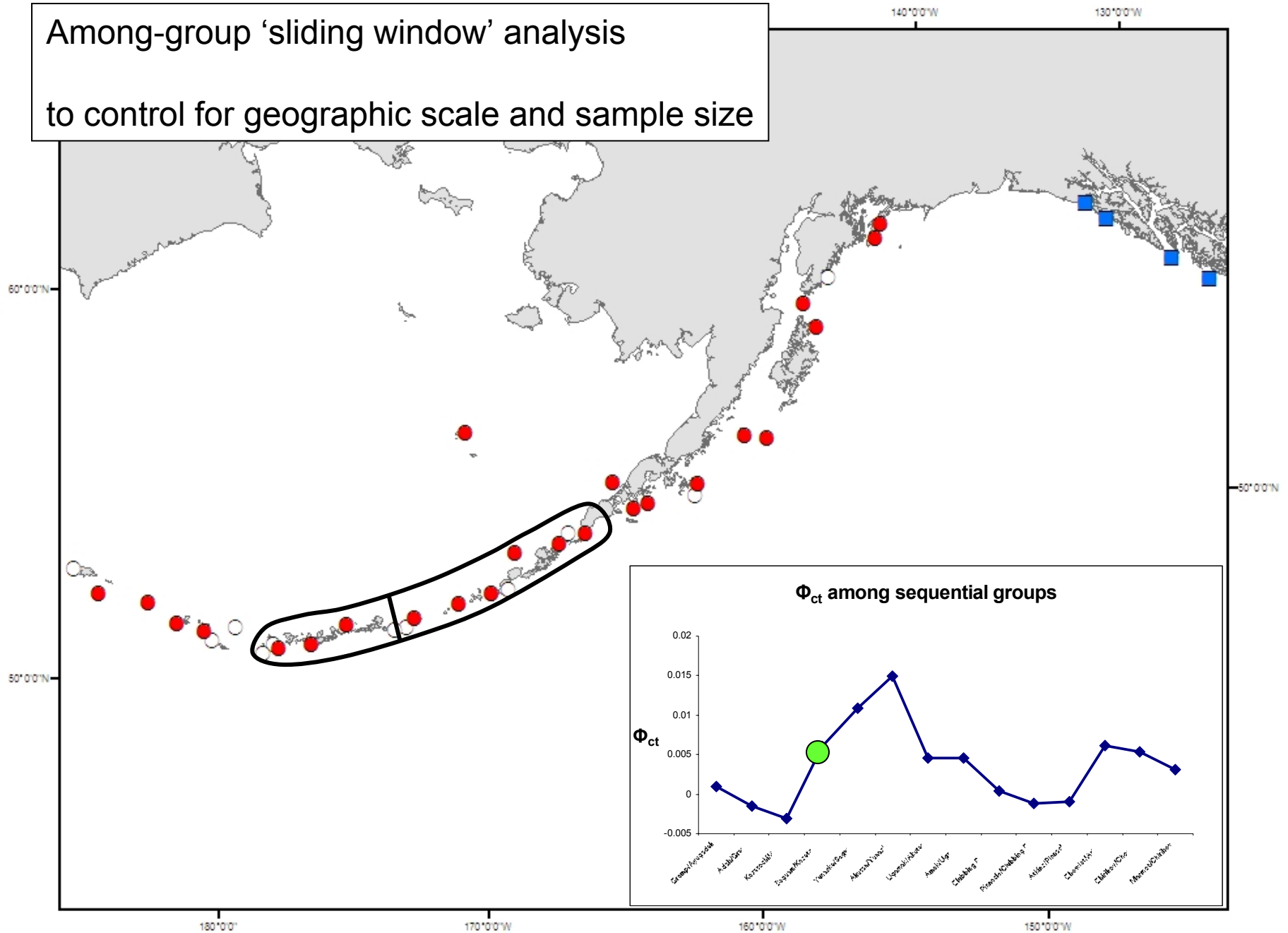
to control for geographic scale and sample size



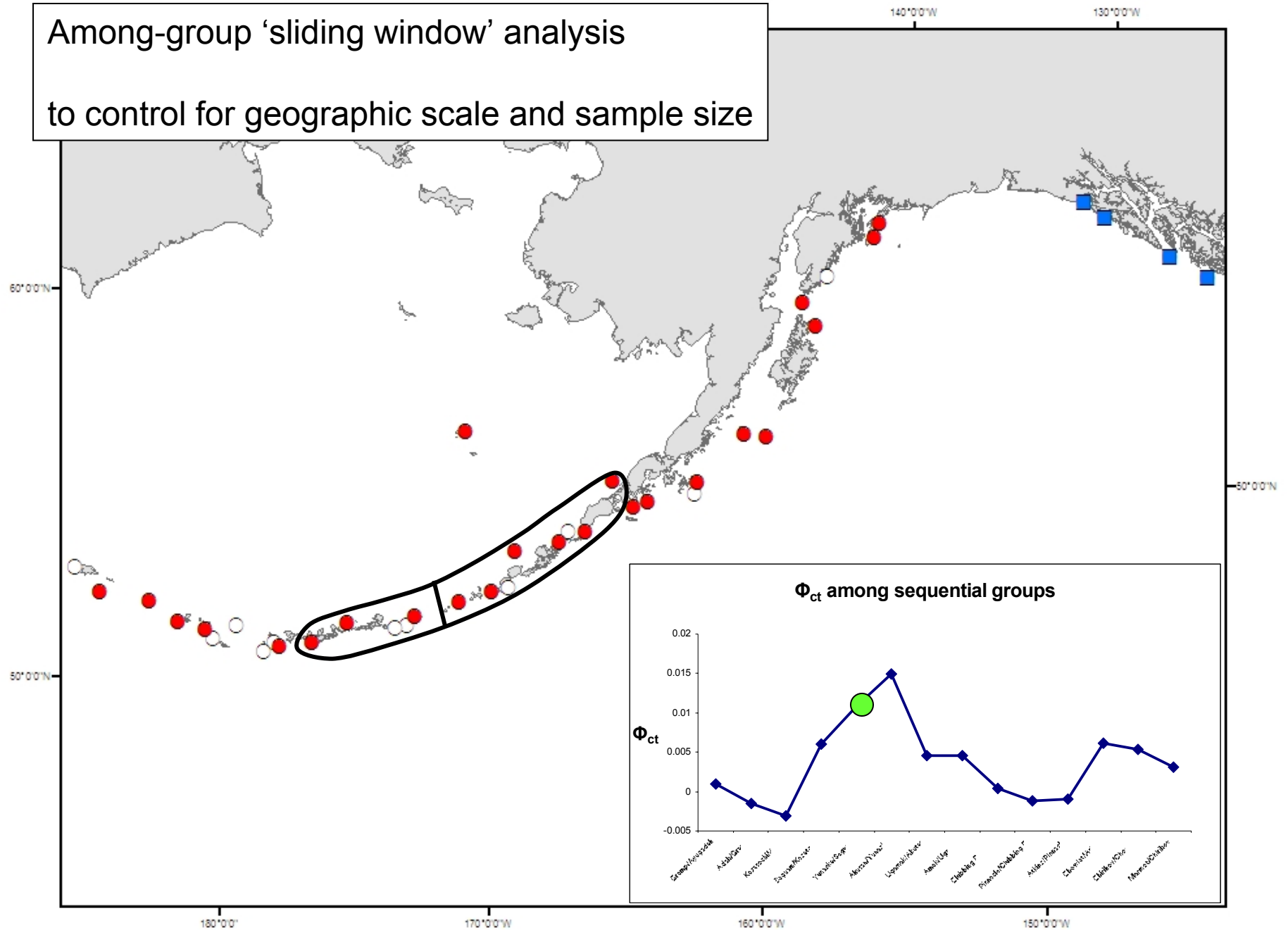
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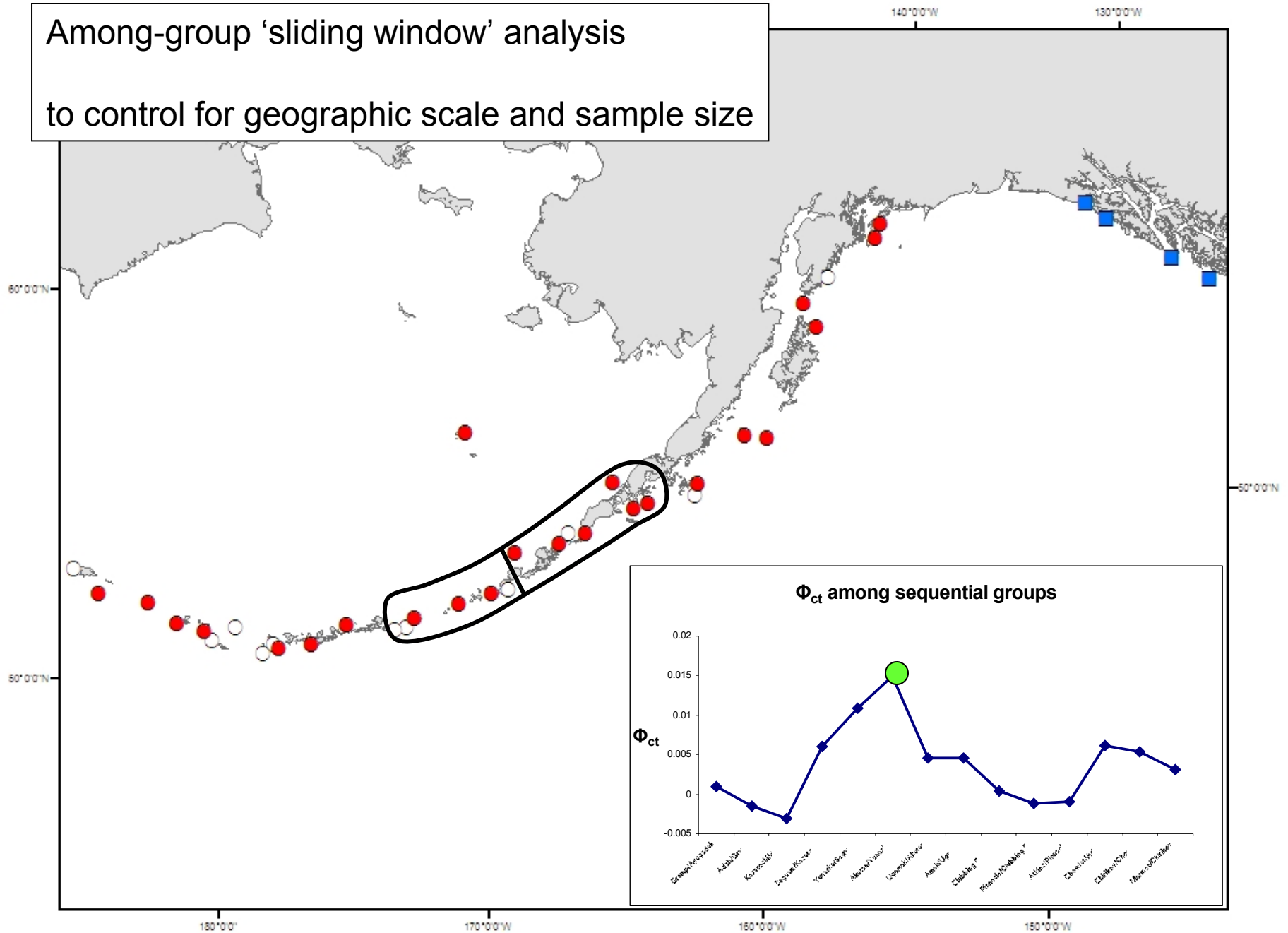
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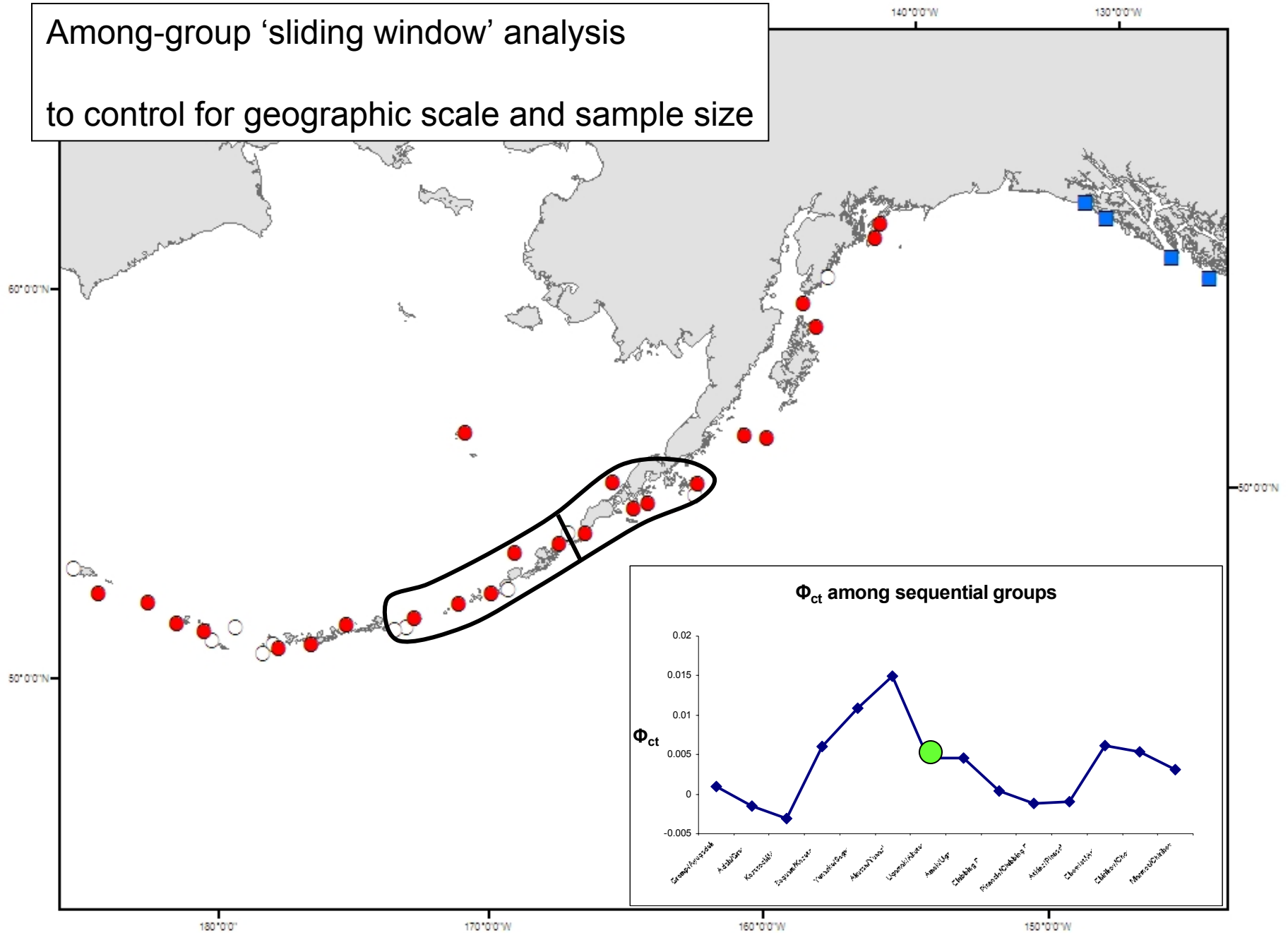
Among-group 'sliding window' analysis
to control for geographic scale and sample size



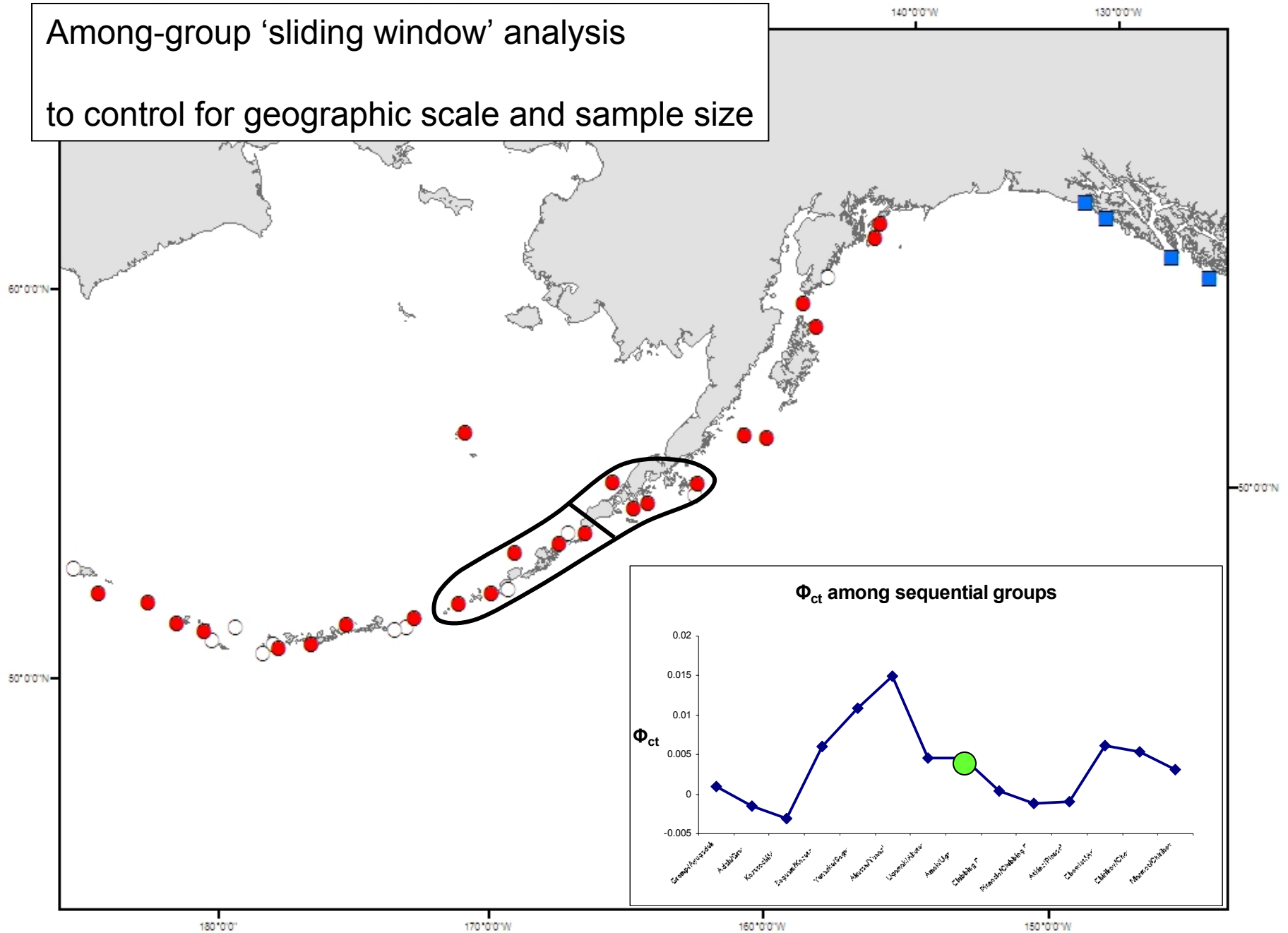
Among-group 'sliding window' analysis
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Among-group 'sliding window' analysis
to control for geographic scale and sample size

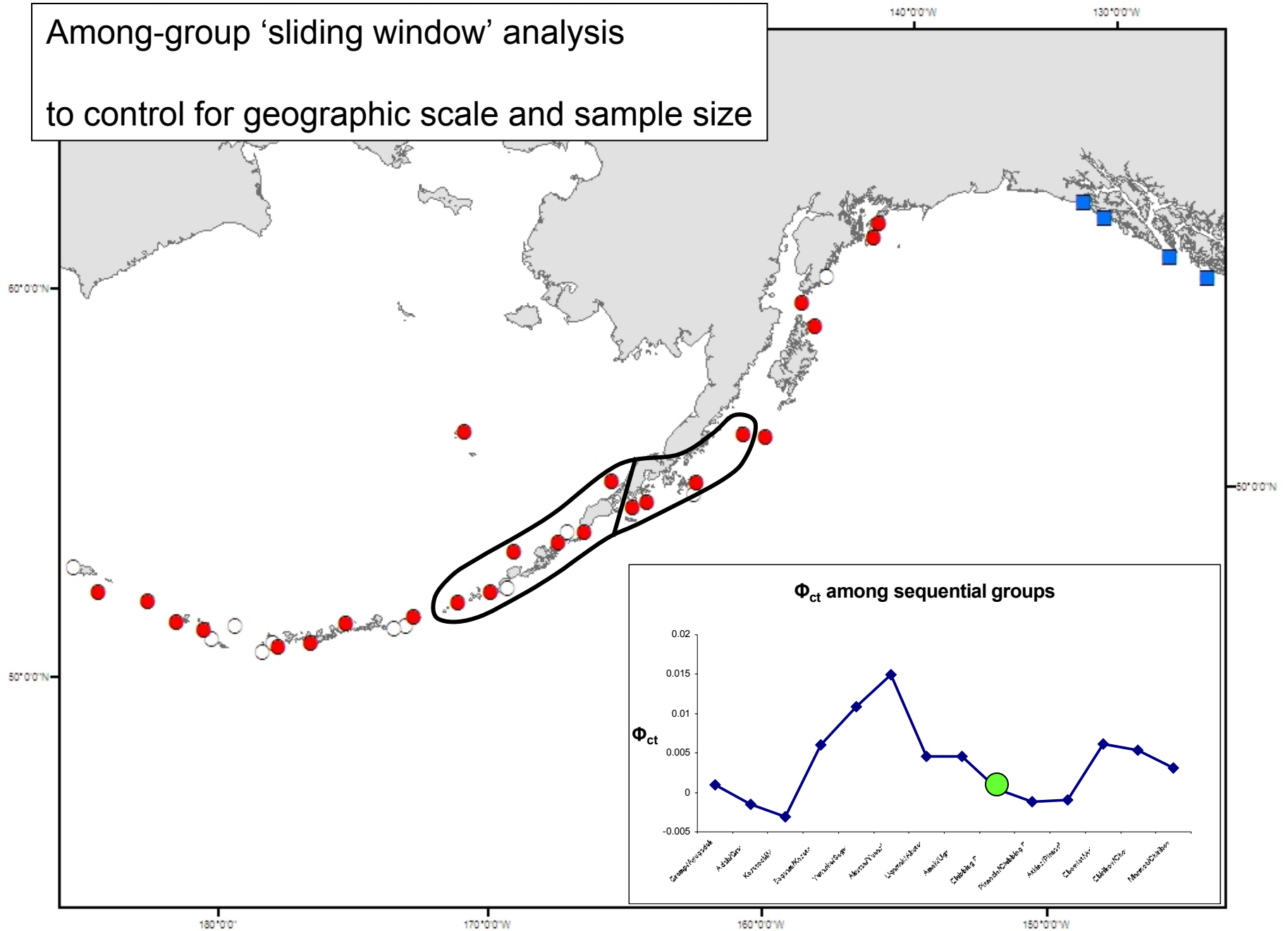


Among-group 'sliding window' analysis
to control for geographic scale and sample size

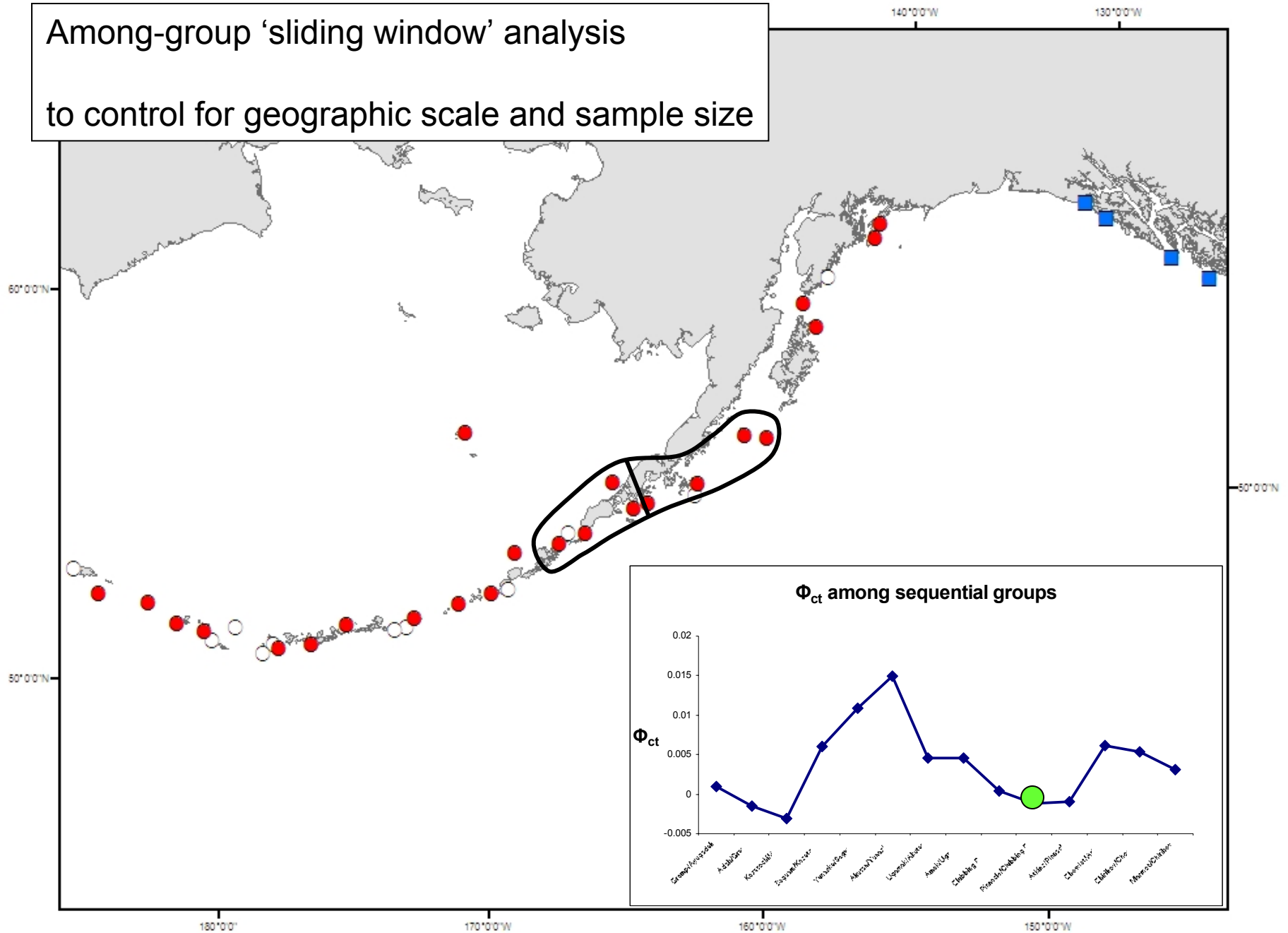


Among-group 'sliding window' analysis

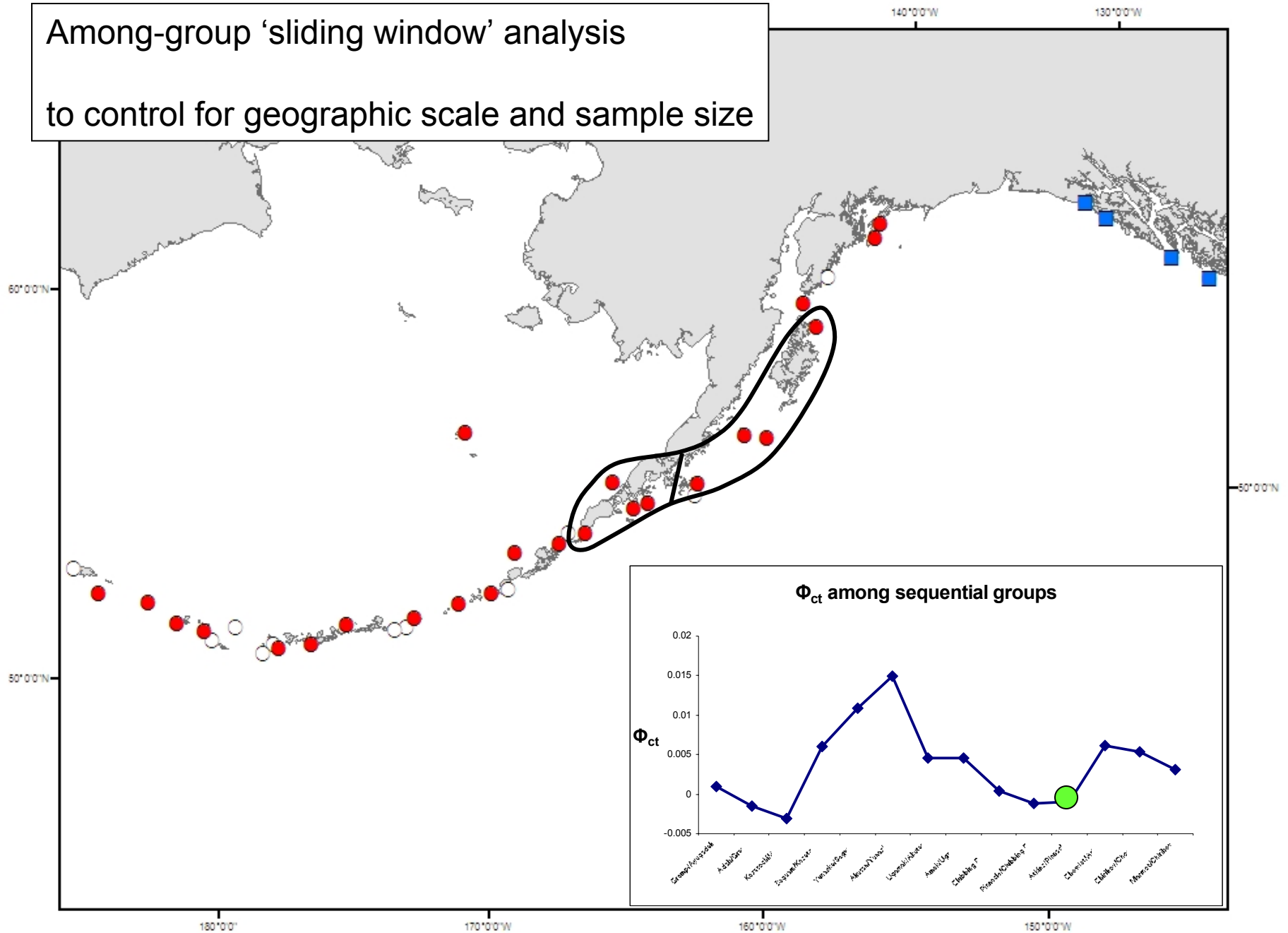
to control for geographic scale and sample size



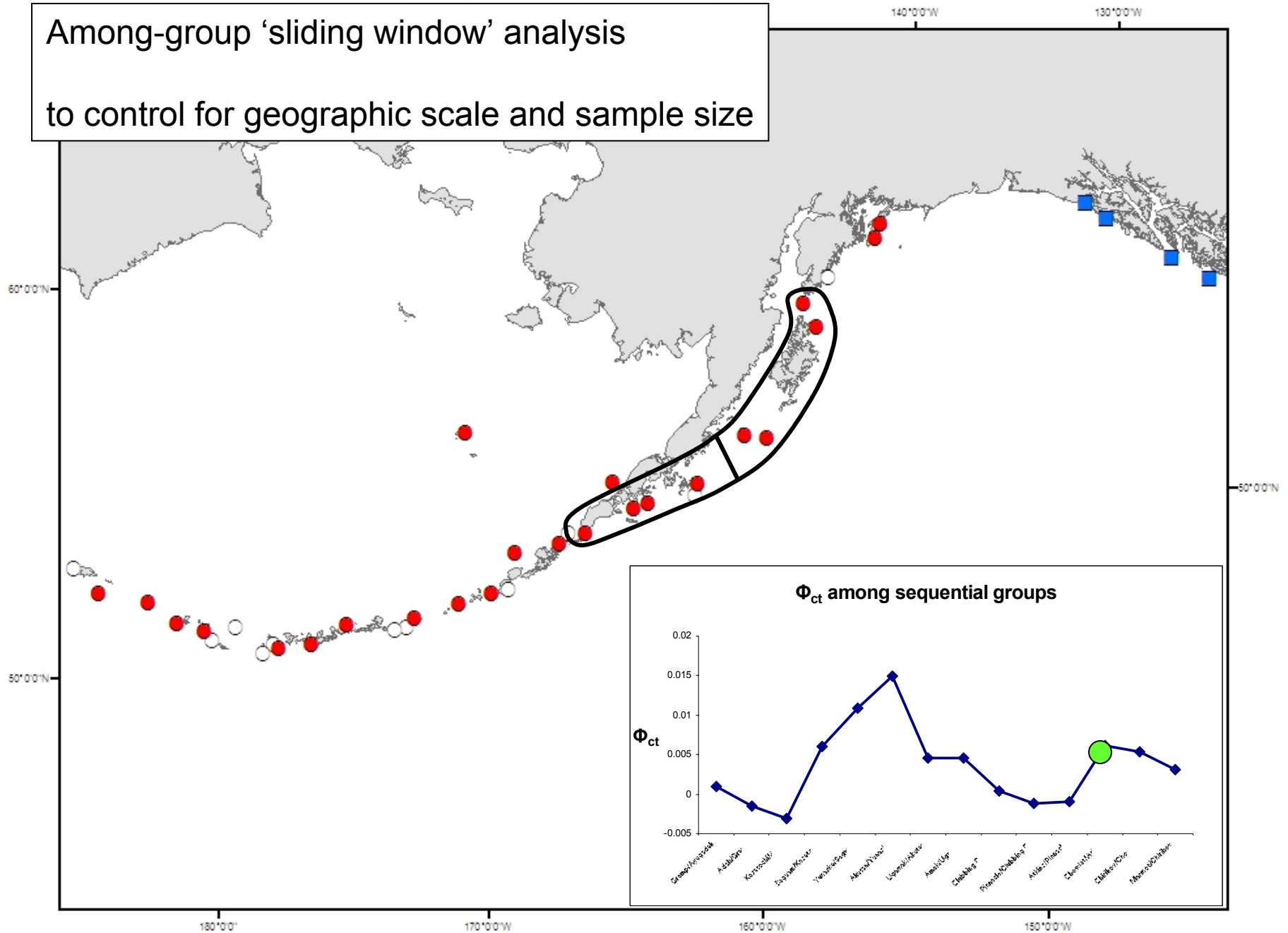
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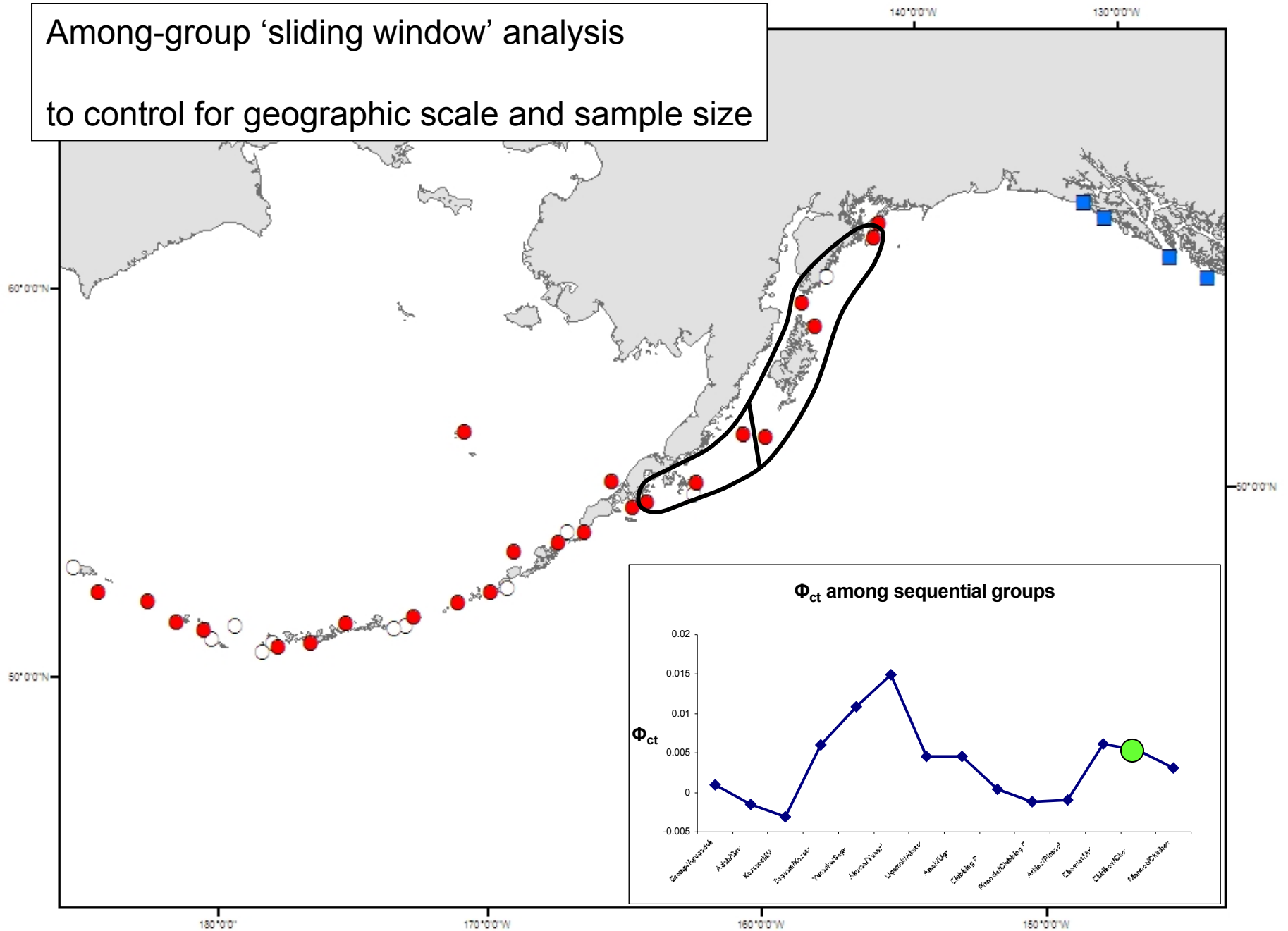


Among-group 'sliding window' analysis
to control for geographic scale and sample size

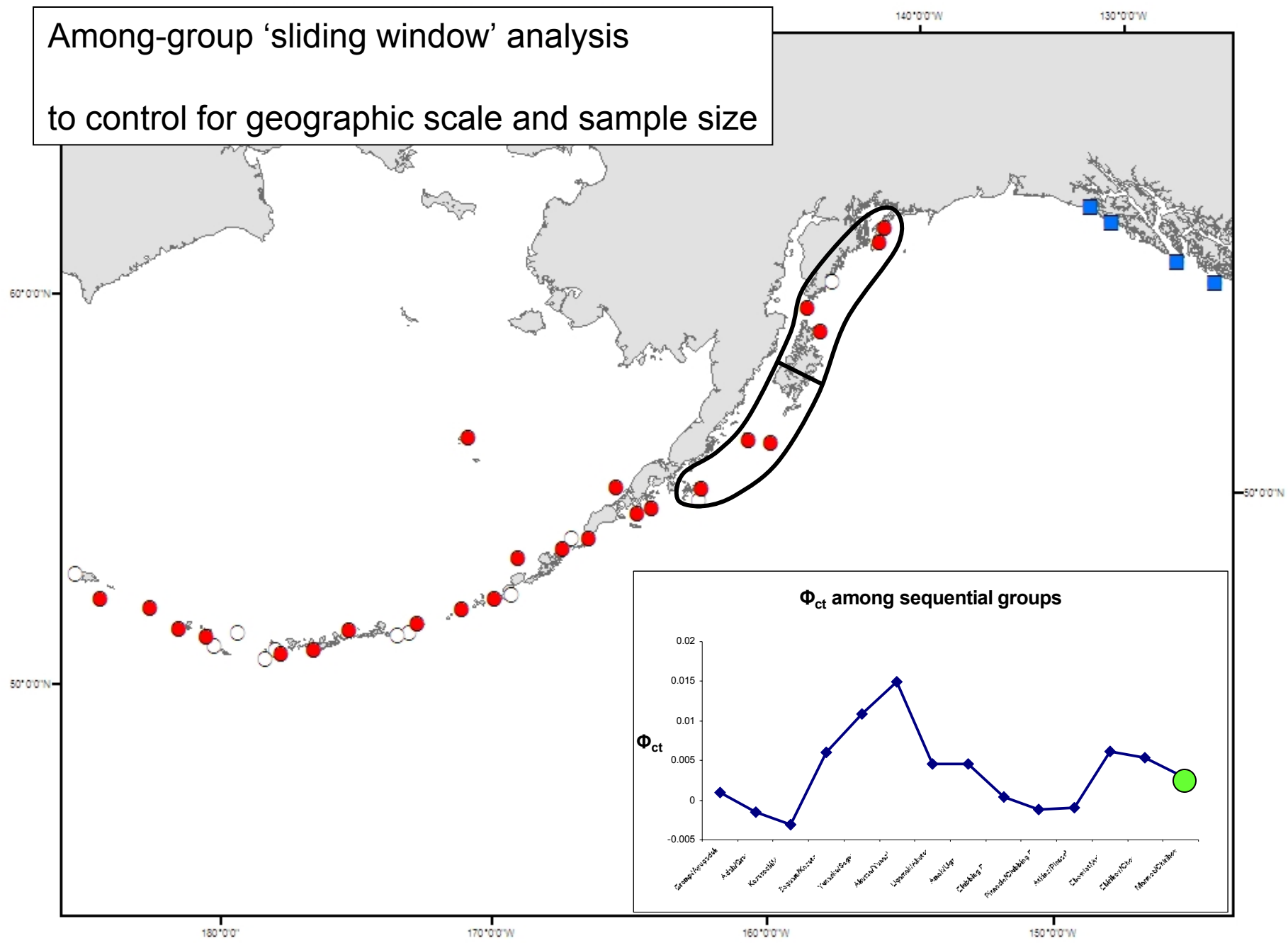


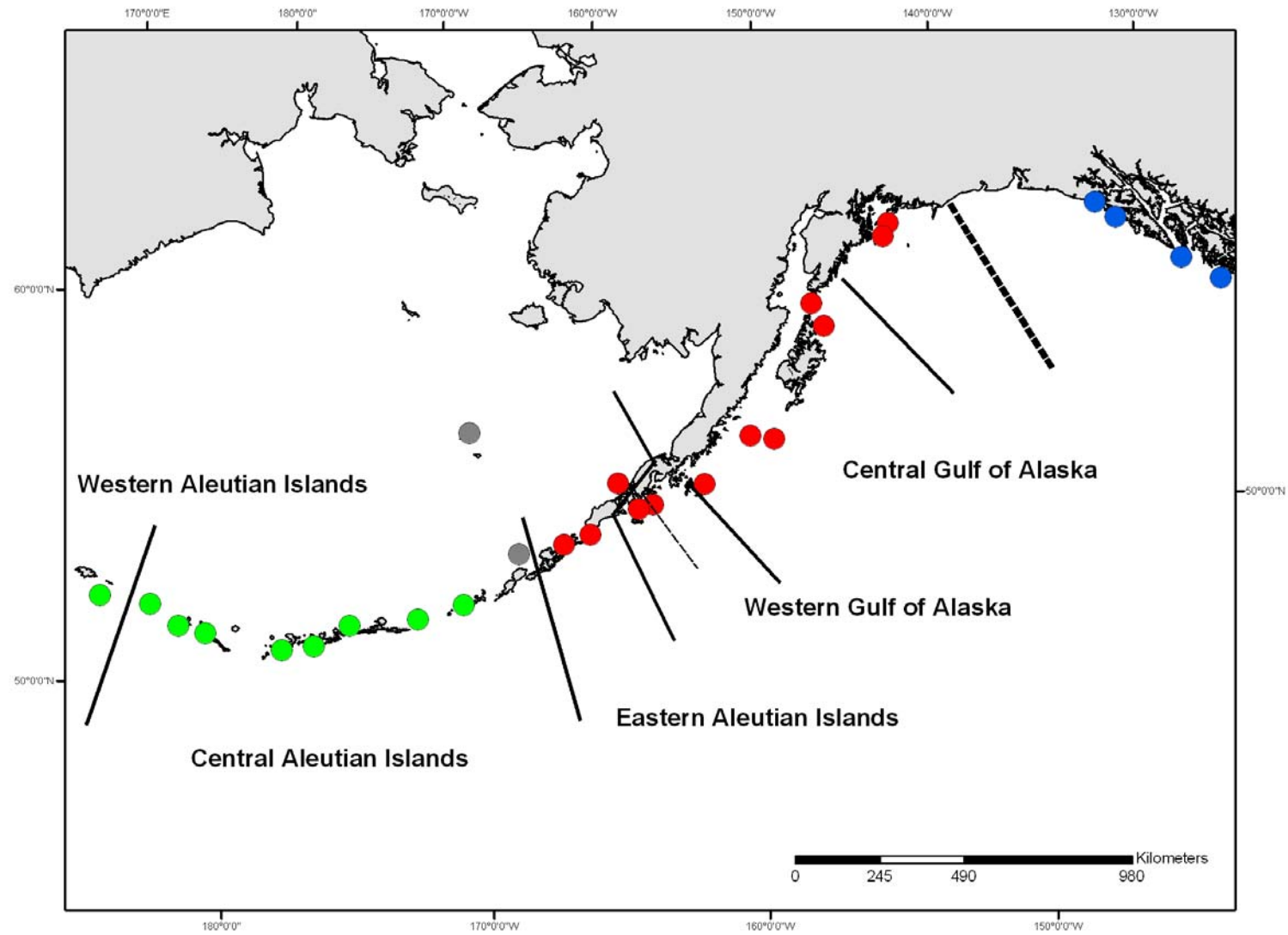
Among-group 'sliding window' analysis

to control for geographic scale and sample size

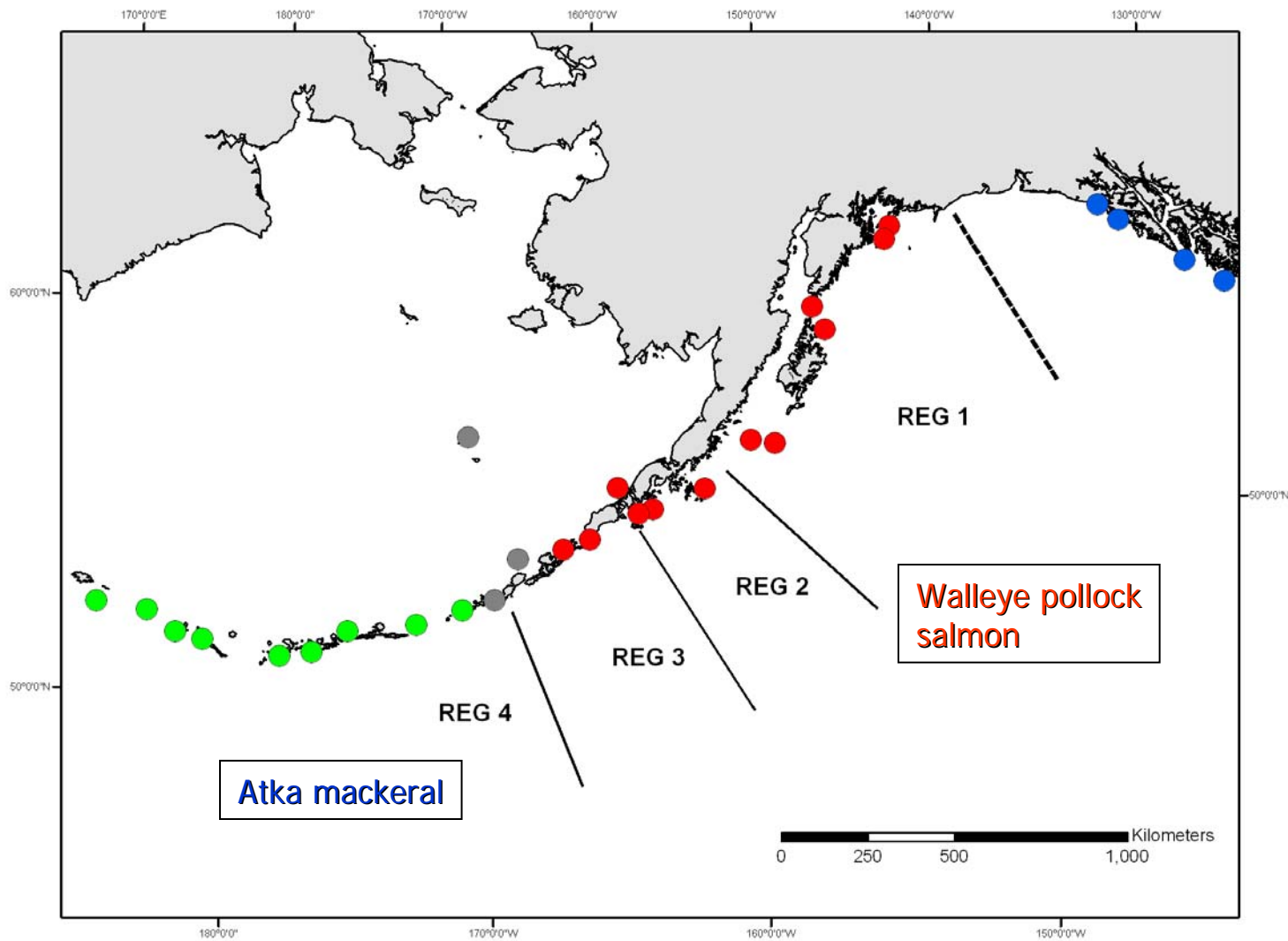


Among-group 'sliding window' analysis
to control for geographic scale and sample size

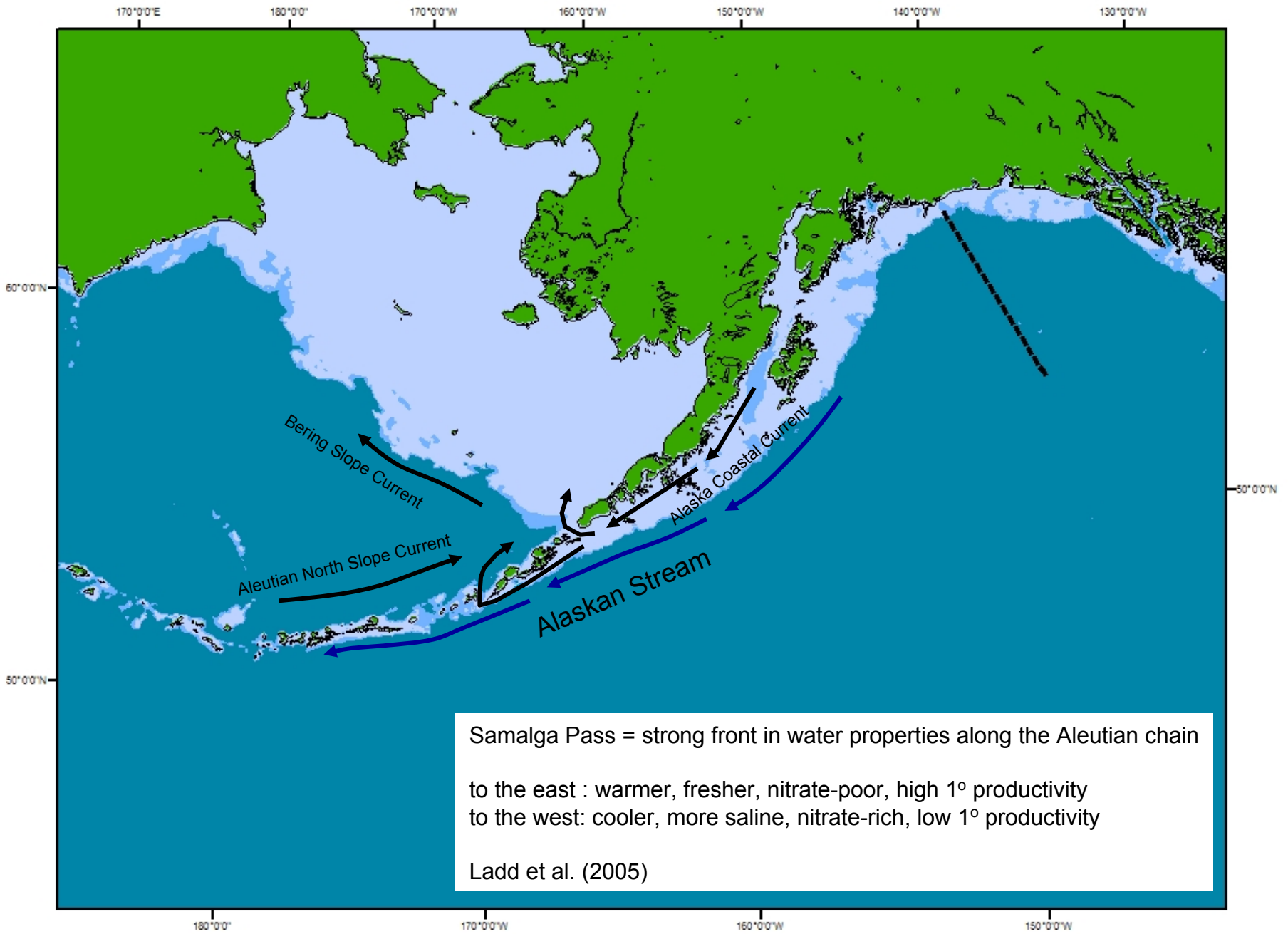


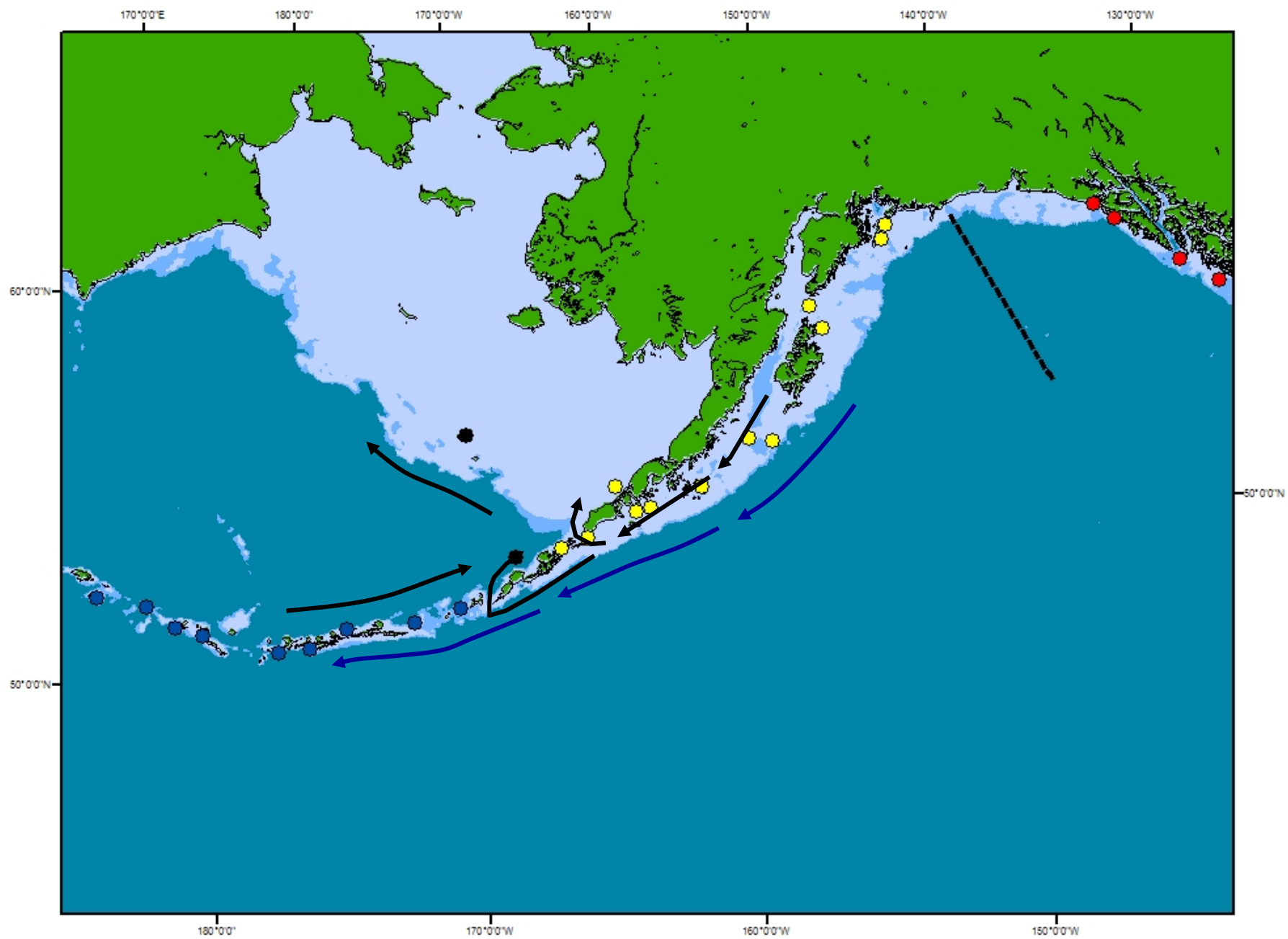


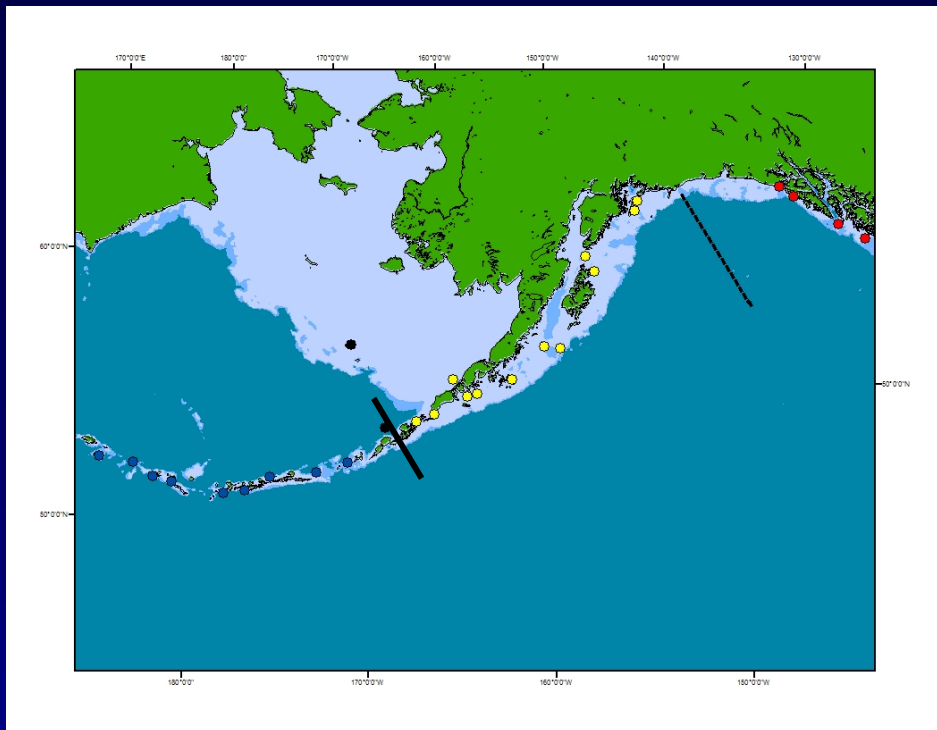
Trends in abundance (York et al., 1996, Sease & Gundmundson, 2002)



Seal lion diet (Sinclair and Zeppelin, 2002)







Conclusions:

The Western DPS of Steller sea lions consists of 2 distinct metapopulations

The split coincides with a strong physical and biogeographic boundary between two marine ecosystems

The 2 metapopulations have separate ecologies and experienced differing trends in abundance

Implications:

Meets the criteria for 2 Distinct Population Segments under U.S. ESA

Future research efforts should be based on this improved understanding of population subdivision and dispersal

Study 2. Emigration, migration and colonization in Steller sea lions: new rookeries suggest a new paradigm

Topic 4: Colonization
Density dependent dispersal
non-equilibrium populations

Key elements:

Assignment tests
Mechanisms of rookery formation
Rare event



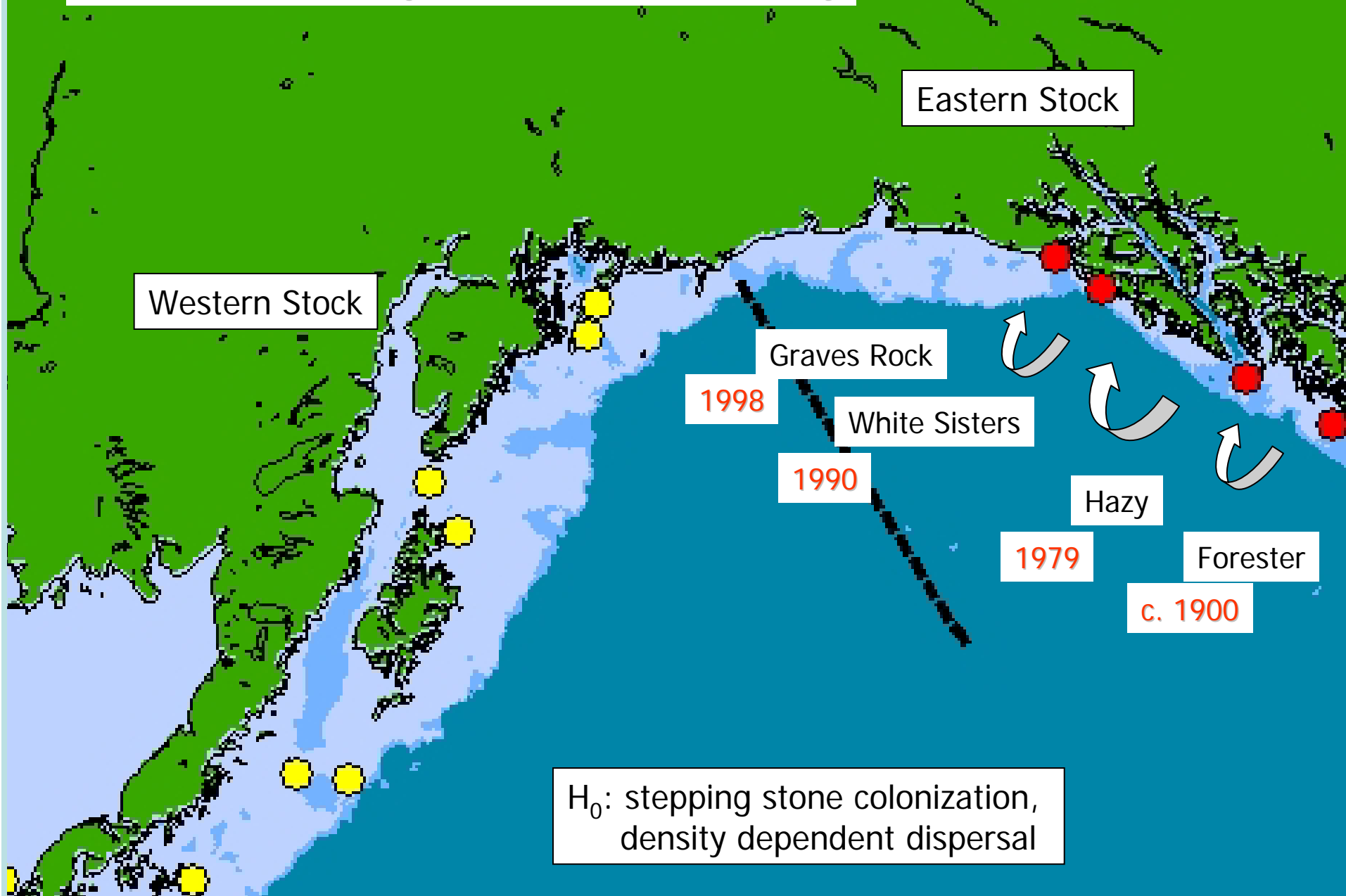
G. O'Corry-Crowe, T. Gelatt, K. Pitcher and B. Taylor (in prep.)



Supported by NOAA Fisheries and the Alaska Department of Fish and Game



Steller sea lion rookery colonization – *mtDNA* study



Eastern Stock

Western Stock

Graves Rock

1998

White Sisters

1990

Hazy



1979

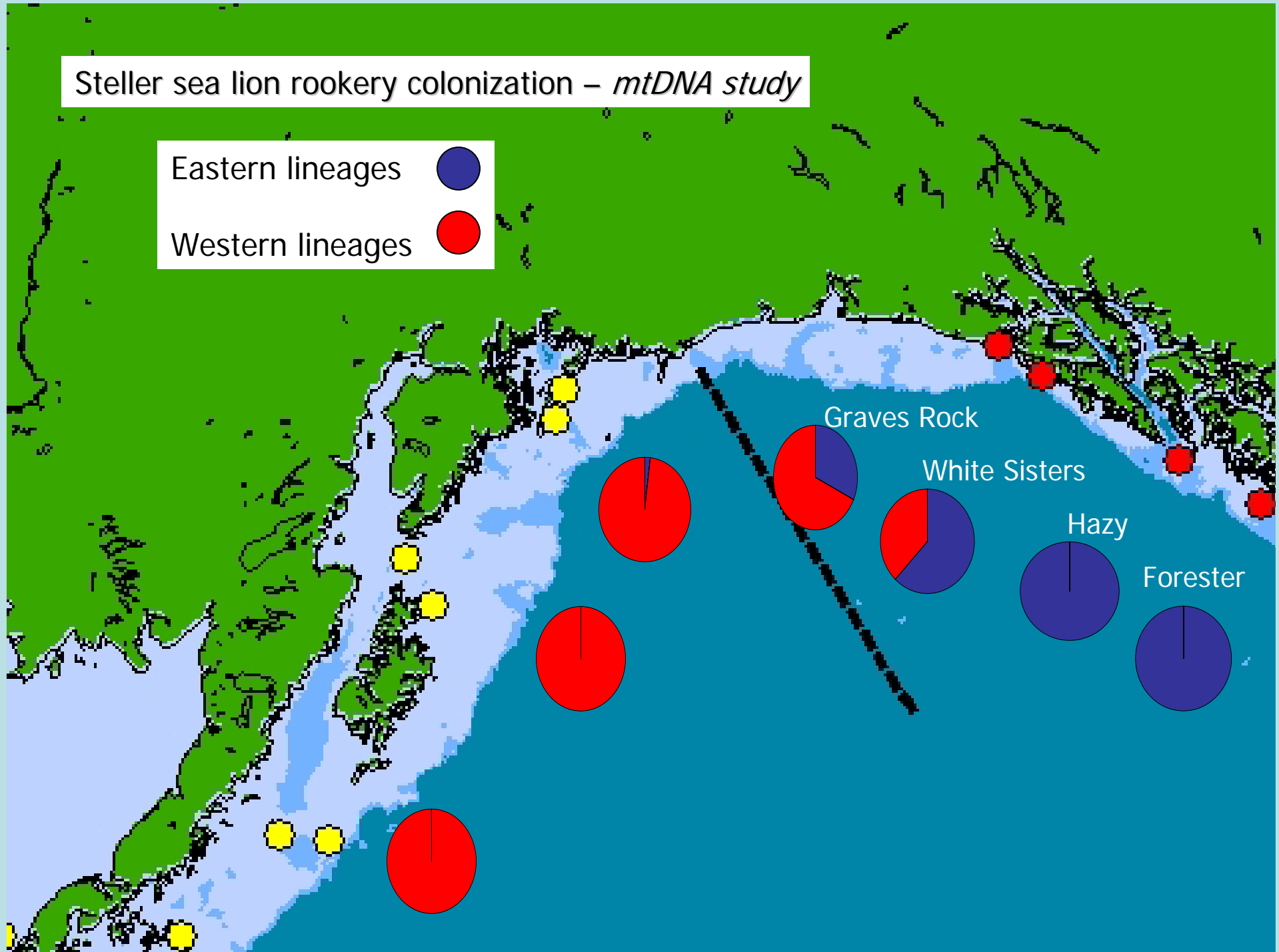
Forester

c. 1900

H_0 : stepping stone colonization,
density dependent dispersal

Steller sea lion rookery colonization – *mtDNA* study

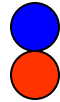
Eastern lineages 
Western lineages 



New sea lion rookeries have mixed origins

Minimum Spanning Tree of 130 mtDNA haplotypes

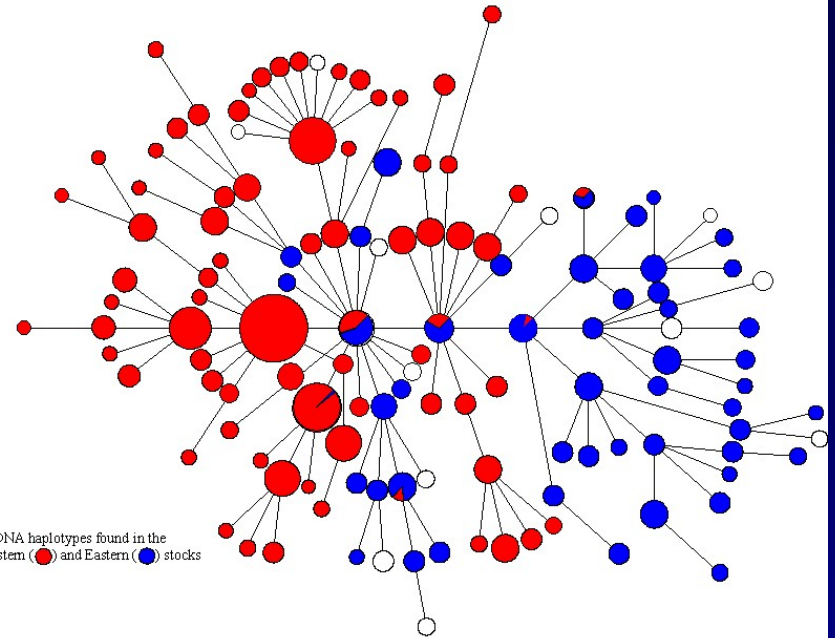
Haplotypes found in the Eastern Stock
Haplotypes found in the Western Stock



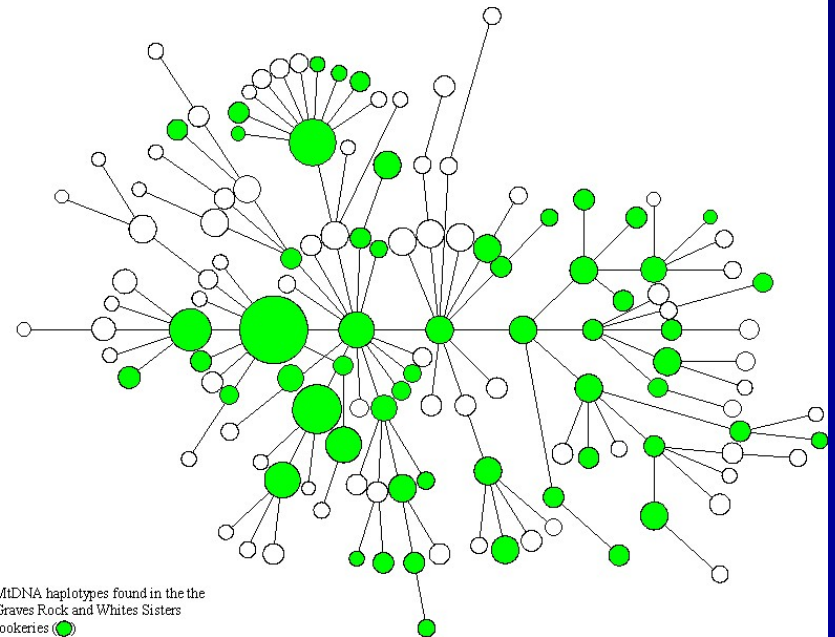
Haplotypes found in Graves Rock and White Sisters



Minimum Spanning Tree of 130 Steller sea lion mtDNA haplotypes



A. mtDNA haplotypes found in the Western (●) and Eastern (●) stocks



B. mtDNA haplotypes found in the Graves Rock and Whites Sisters rookeries (●)

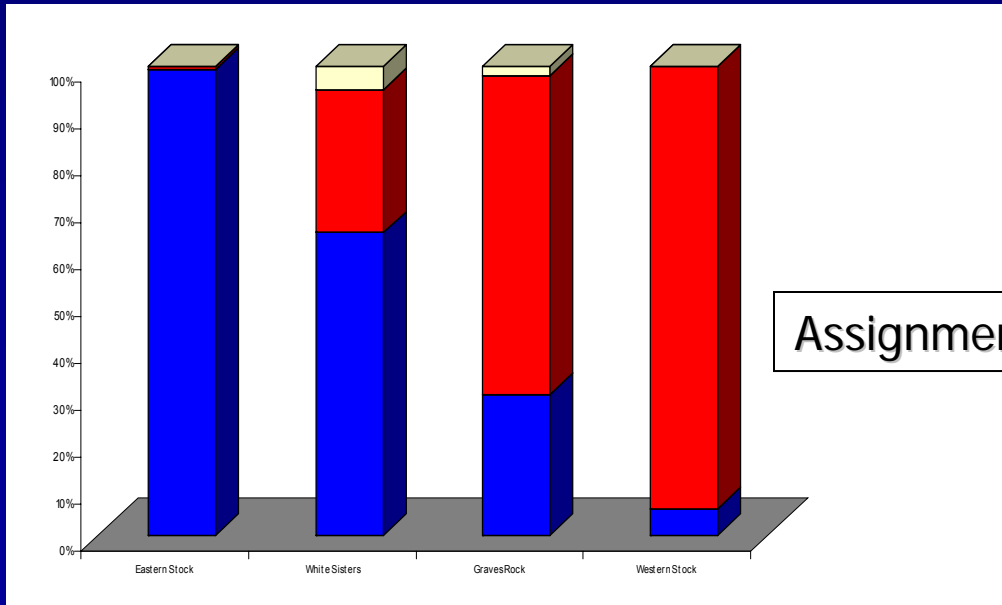
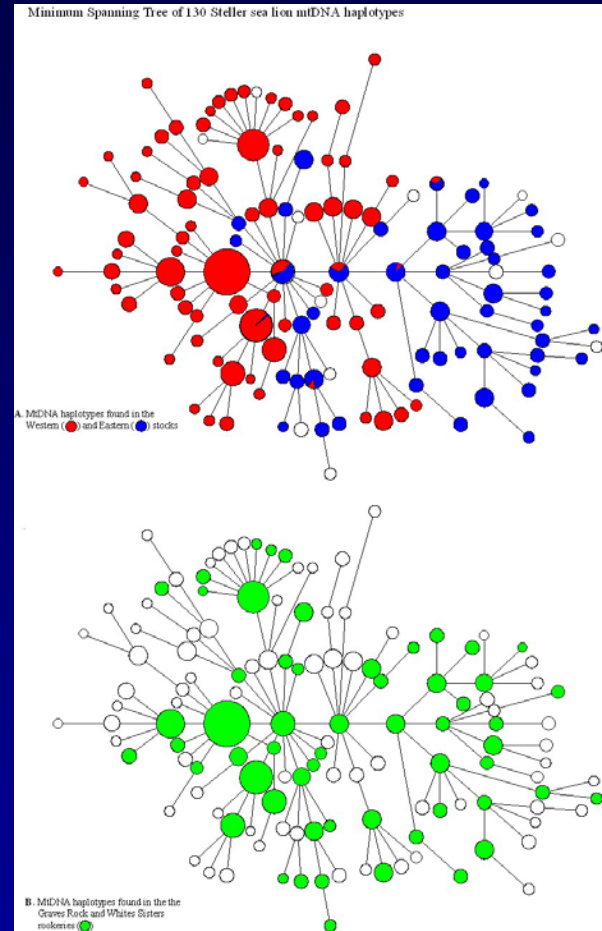
New sea lion rookeries have mixed origins

Minimum Spanning Tree of 130 mtDNA haplotypes

Haplotypes found in the Eastern Stock
 Haplotypes found in the Western Stock

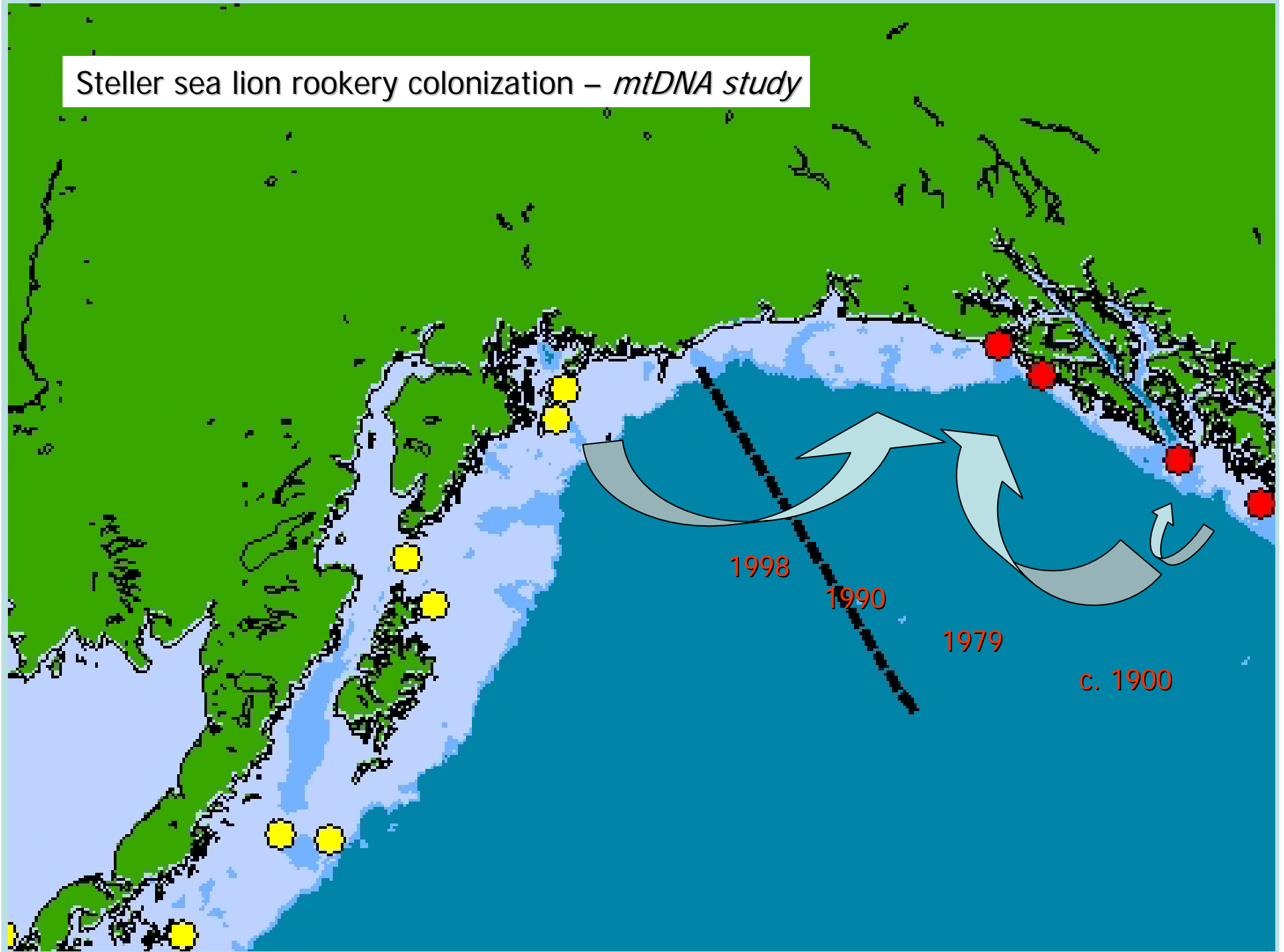


Haplotypes found in Graves Rock and White Sisters



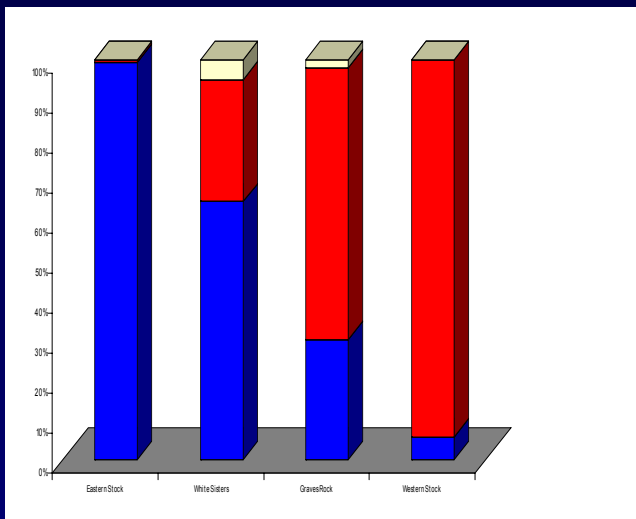
Assignment test

Steller sea lion rookery colonization – *mtDNA* study



Branded female from Sugarloaf on White Sisters with a newborn pup





Conclusions:

Molecular genetics documented behaviour in real time

Molecular genetics documented a rare event, emigration across evolutionarily distinct boundaries.

Motivations for dispersal likely differed between females from the Western DPS and the Eastern DPS

A unique opportunity to document the mechanisms of rookery formation

Implications:

Questions our understanding of the current stock boundaries

Interpretation of comparative studies may need to be re-evaluated

Caution when applying equilibrium models to population structure inference.

Future directions:

Investigating the male component by screening 40 microsatellite markers

Study 3. **Kinship, mating systems and colonization in Steller sea lions**

Topic 4: Relatedness
Paternity
Colonization

Key elements:

DNA fingerprinting
Assignment tests
Mechanisms of rookery formation
Rare event



G. O'Corry-Crowe, C. Bonin, T. Gelatt, K. Pitcher (In data generation and analysis phase)



Supported by NOAA Fisheries and the Alaska Department of Fish and Game



Population Structure, kinship, mating systems and dispersal patterns in Steller Sea lions

Marker type: microsatellites
37 tested
n=574
12 rookeries

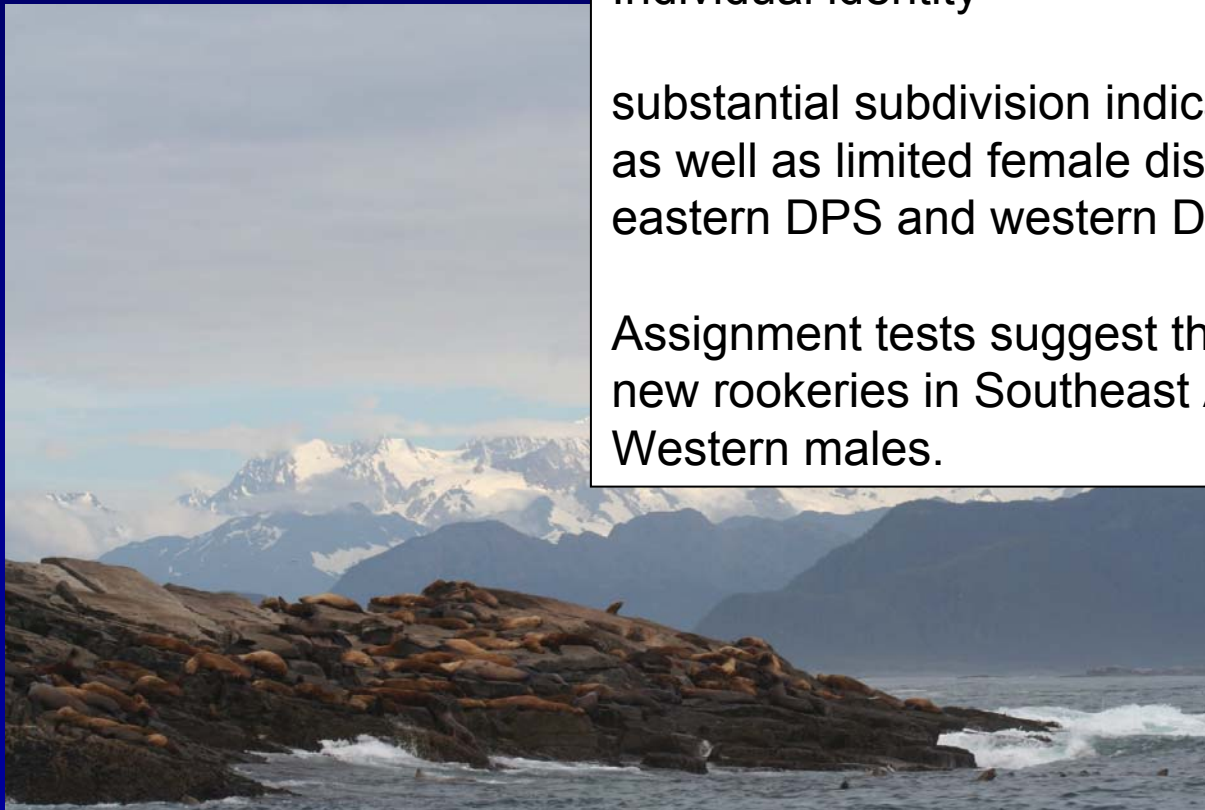
Preliminary results:

10 hypervariable loci screened for 574 sea lions
7 more to screen

Individual identity

substantial subdivision indicating limited interbreeding
as well as limited female dispersal between the
eastern DPS and western DPS

Assignment tests suggest that some pups born on
new rookeries in Southeast Alaska were fathered by
Western males.



Future Directions

Microsatellite analysis of the Aleutian Passes break

Genetics of colonization and extinction on other rookeries

Steller sea lion mating systems

Paternity of pups on White Sisters and Graves Rocks rookeries

Structure within rookeries



Acknowledgements

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